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(54) Title: GROWTH DIFFERENTIATION FACTOR-8 (57) Abstract A transgenic non-human animal of the species selected from the group consisting of avian, bovine, ovine and porcine having a transgene which results in disrupting the production of and/or activity of growth differentiation factor-8 (GDF-8) chromosomally integrated into the germ cells of the animal is disclosed. Also disclosed are methods for making such animals, and methods of treating animals, including humans, with antibodies or antisense directed to GDF-8. The animals so treated are characterized by increased muscle tissue and bone content.		

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GROWTH DIFFERENTIATION FACTOR-8

BACKGROUND OF THE INVENTION

5 1. Field of the Invention

The invention relates generally to growth factors and specifically to a new member of the transforming growth factor beta (TGF- β) superfamily, which is denoted, growth differentiation factor-8 (GDF-8) and methods of use for modulating muscle, bone, kidney and adipose cell and tissue growth.

10 2. Description of Related Art

The transforming growth factor β (TGF- β) superfamily encompasses a group of structurally-related proteins which affect a wide range of differentiation processes during embryonic development. The family includes, Mullerian inhibiting substance (MIS), which is required for normal male sex development (Behringer, *et al.*, *Nature*, 345:167, 15 1990), Drosophila decapentaplegic (DPP) gene product, which is required for dorsal-ventral axis formation and morphogenesis of the imaginal disks (Padgett, *et al.*, *Nature*, 325:81 -84, 1987), the Xenopus Vg-1 gene product, which localizes to the vegetal pole of eggs ((Weeks, *et al.*, *Cell*, 51:861-867, 1987), the activins (Mason, *et al.*, *Biochem, Biophys. Res. Commun.*, 135:957-964, 1986), which can induce the formation 20 of mesoderm and anterior structures in Xenopus embryos (Thomsen, *et al.*, *Cell*, 63:485, 1990), and the bone morphogenetic proteins (BMPs, osteogenin, OP-1) which can induce de novo cartilage and bone formation (Sampath, *et al.*, *J. Biol. Chem.*, 265:13198, 1990). The TGF- β s can influence a variety of differentiation processes, including adipogenesis, myogenesis, chondrogenesis, hematopoiesis, and epithelial cell differentiation (for 25 review, see Massague, *Cell* 49:437, 1987).

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The proteins of the TGF- β family are initially synthesized as a large precursor protein which subsequently undergoes proteolytic cleavage at a cluster of basic residues approximately 110-140 amino acids from the C-terminus. The C-terminal regions, or mature regions, of the proteins are all structurally related and the different family members can be classified into distinct subgroups based on the extent of their homology. Although the homologies within particular subgroups range from 70% to 90% amino acid sequence identity, the homologies between subgroups are significantly lower, generally ranging from only 20% to 50%. In each case, the active species appears to be a disulfide-linked dimer of C-terminal fragments. Studies have shown that when the pro-region of a member of the TGF- β family is coexpressed with a mature region of another member of the TGF- β family, intracellular dimerization and secretion of biologically active homodimers occur (Gray, A. *et al.*, *Science*, 247:1328, 1990). Additional studies by Hammonds, *et al.*, (*Molec. Endocrin.* 5:149, 1991) showed that the use of the BMP-2 pro-region combined with the BMP-4 mature region led to dramatically improved expression of mature BMP-4. For most of the family members that have been studied, the homodimeric species has been found to be biologically active, but for other family members, like the inhibins (Ling, *et al.*, *Nature*, 321 :779, 1986) and the TGF- β s (Cheifetz, *et al.*, *Cell*, 48:409, 1987), heterodimers have also been detected, and these appear to have different biological properties than the respective homodimers.

20

In addition it is desirable to produce livestock and game animals, such as cows, sheep, pigs, chicken and turkey, fish which are relatively high in musculature and protein, and low in fat content. Many drug and diet regimens exist which may help increase muscle and protein content and lower undesirably high fat and/or cholesterol levels, but such treatment is generally administered after the fact, and is begun only after significant damage has occurred to the vasculature. Accordingly, it would be desirable to produce animals which are genetically predisposed to having higher muscle and/or bone content, without any ancillary increase in fat levels.

The food industry has put much effort into increasing the amount of muscle and protein in foodstuffs. This quest is relatively simple in the manufacture of synthetic foodstuffs but has been met with limited success in the preparation of animal foodstuffs. Attempts have been made, for example, to lower cholesterol levels in beef and poultry products by including cholesterol-lowering drugs in animal feed (see *e.g.* Elkin and Rogler, J. Agric. Food Chem. 1990, 38, 1635-1641). However, there remains a need for more effective methods of increasing muscle and reducing fat and cholesterol levels in animal food products.

10 The present invention provides a cell growth and differentiation factor, GDF-8, a polynucleotide sequence which encodes the factor, and antibodies which are immunoreactive with the factor. This factor appears to relate to various cell proliferative disorders, especially those involving muscle, nerve, bone, kidney and adipose tissue.

In one embodiment, the invention provides a method for detecting a cell proliferative disorder of muscle, nerve, bone, kidney or fat origin and which is associated with GDF-8. In another embodiment, the invention provides a method for treating a cell proliferative disorder by suppressing or enhancing GDF-8 activity.

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bone content. The transgenic non-human animals of the invention include bovine, porcine, ovine and avian animals, for example.

The subject invention also provides a method of producing animal food products having increased bone content. The method includes modifying the genetic makeup of the germ cells of a pronuclear embryo of the animal, implanting the embryo into the oviduct of a pseudopregnant female thereby allowing the embryo to mature to full term progeny, testing the progeny for presence of the transgene to identify transgene-positive progeny, cross-breeding transgene-positive progeny to obtain further transgene-positive progeny and processing the progeny to obtain foodstuff. The modification of the germ cell comprises altering the genetic composition so as to disrupt or reduce the expression of the naturally occurring gene encoding for production of GDF-8 protein. In a particular embodiment, the transgene comprises antisense polynucleotide sequences to the GDF-8 protein. Alternatively, the transgene may comprise a non-functional sequence which replaces or intervenes in the native GDF-8 gene.

The subject invention also provides a method of producing avian food products having improved muscle and/or bone content. The method includes modifying the genetic makeup of the germ cells of a pronuclear embryo of the avian animal, implanting the embryo into the oviduct of a pseudopregnant female into an embryo of a chicken, culturing the embryo under conditions whereby progeny are hatched, testing the progeny for presence of the genetic alteration to identify transgene-positive progeny, cross-breeding transgene-positive progeny and processing the progeny to obtain foodstuff.

The invention also provides a method for treating a muscle, bone, kidney or adipose tissue disorder in a subject. The method includes administering a therapeutically effective amount of a GDF-8 agent to the subject, thereby inhibiting abnormal growth of muscle, bone or adipose tissue. The GDF-8 agent may include an antibody, a GDF-8 antisense molecule or a dominant negative polypeptide, for example. In one aspect, a method for inhibiting the growth regulating actions of GDF-8 by contacting an anti-

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GDF-8 monoclonal antibody, a GDF-8 antisense molecule or a dominant negative polypeptide (or polynucleotide encoding a dominant negative polypeptide) with fetal or adult muscle cells, bone cells or progenitor cells is included. These agents can be administered to a patient suffering from a disorder such as muscle wasting disease, neuromuscular disorder, muscle atrophy, osteoporosis, bone degenerative diseases, obesity or other adipocyte cell disorders, and aging, for example. In another aspect of the invention, the agent may be an agonist of GDF-8 activity. In this embodiment, the agonist may be administered to promote kidney cell growth and differentiation in kidney tissue.

- 10 The invention also provides a method for identifying a compound that affects GDF-8 activity or gene expression including incubating the compound with GDF-8 polypeptide, or with a recombinant cell expressing GDF-8 under conditions sufficient to allow the compounds to interact and determining the effect of the compound on GDF-8 activity or expression.

15 **BRIEF DESCRIPTION OF THE DRAWINGS**

FIGURE 1a is a Northern blot showing expression of GDF-8 mRNA in adult tissues. The probe was a partial murine GDF-8 clone.

FIGURE 1b is a Southern blot showing GDF-8 genomic sequences identified in mouse, rat, human, monkey, rabbit, cow, pig, dog and chicken.

- 20 FIGURE 2 shows partial nucleotide and predicted amino acid sequences of murine GDF-8 (FIGURE 2a; SEQ ID NO:11 and 12, respectively), human GDF-8 (FIGURE 2b; SEQ ID NO: 13 and 14, respectively), rat GDF-8 (FIGURE 2c; SEQ ID NO: 24 and 25, respectively) and chicken GDF-8 (FIGURE 2d; SEQ ID NO: 22 and 23, respectively). The putative dibasic processing sites in the murine sequence are boxed.

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FIGURE 3a shows the alignment of the C-terminal sequences of GDF-8 with other members of the TGF- β superfamily. The conserved cysteine residues are boxed. Dashes denote gaps introduced in order to maximize alignment.

FIGURE 3b shows the alignment of the C-terminal sequences of GDF-8 from human,
5 murine, rat and chicken sequences.

FIGURE 4 shows amino acid homologies among different members of the TGF superfamily. Numbers represent percent amino acid identities between each pair calculated from the first conserved cysteine to the C-terminus. Boxes represent homologies among highly-related members within particular subgroups.

10 FIGURE 5 shows the sequence of GDF-8. Nucleotide and amino acid sequences of murine (FIGURE 5a and 5b)(GenBank accession number U84005; SEQ ID NO:11 and 12, respectively) and human (FIGURE 5c and 5d; SEQ ID NO:13 and 14, respectively) GDF-8 cDNA clones are shown. Numbers indicate nucleotide position relative to the 5' end. Consensus N-linked glycosylation signals are shaded. The putative RXXR
15 proteolytic cleavage sites are boxed.

FIGURE 6 shows a hydropathicity profile of GDF-8. Average hydrophobicity values for murine (FIGURE 6a) and human (FIGURE 6b) GDF-8 were calculated using the method of Kyte and Doolittle (*J. Mol. Biol.*, 157:105-132, 1982). Positive numbers indicate increasing hydrophobicity.

20 FIGURE 7 shows a comparison of murine and human GDF-8 amino acid sequences. The predicted murine sequence is shown in the top lines and the predicted human sequence is shown in the bottom lines. Numbers indicate amino acid position relative to the N-terminus. Identities between the two sequences are denoted by a vertical line.

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FIGURE 8 shows the expression of GDF-8 in bacteria. BL21 (DE3) (pLysS) cells carrying a pRSET/GDF-8 expression plasmid were induced with isopropylthio- β -galactoside, and the GDF-8 fusion protein was purified by metal chelate chromatography. Lanes: total=total cell lysate; soluble=soluble protein fraction; insoluble=insoluble protein fraction (resuspended in 10 mM Tris pH 8.0, 50 mM sodium phosphate, 8 M urea, and 10 mM β -mercaptoethanol [buffer B]) loaded onto the column; pellet=insoluble protein fraction discarded before loading the column; flowthrough=proteins not bound by the column; washes=washes carried out in buffer B at the indicated pH's. Positions of molecular weight standards are shown at the right.

10 Arrow indicates the position of the GDF-8 fusion protein.

FIGURE 9 shows the expression of GDF-8 in mammalian cells. Chinese hamster ovary cells were transfected with pMSXND/GDF-8 expression plasmids and selected in G418. Conditioned media from G418-resistant cells (prepared from cells transfected with constructs in which GDF-8 was cloned in either the antisense or sense orientation) were concentrated, electrophoresed under reducing conditions, blotted, and probed with anti-GDF-8 antibodies and [125 I]iodoproteinA. Arrow indicates the position of the processed GDF-8 protein.

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FIGURE 10 shows the expression of GDF-8 mRNA. Poly A-selected RNA (5 μ g each) prepared from adult tissues (FIGURE 10a) or placentas and embryos (FIGURE 10b) at the indicated days of gestation was electrophoresed on formaldehyde gels, blotted, and probed with full length murine GDF-8.

20

FIGURE 11 shows chromosomal mapping of human GDF-8. DNA samples prepared from human/rodent somatic cell hybrid lines were subjected to PCR, electrophoresed on agarose gels, blotted, and probed. The human chromosome contained in each of the hybrid cell lines is identified at the top of each of the first 24 lanes (1-22, X, and Y). In the lanes designated M, CHO, and H, the starting DNA template was total genomic DNA

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from mouse, hamster, and human sources, respectively. In the lane marked B1, no template DNA was used. Numbers at left indicate the mobilities of DNA standards.

Figure 12a shows a map of the GDF-8 locus (top line) and targeting construct (second line). The black and stippled boxes represent coding sequences for the pro- and C-terminal regions, respectively. The white boxes represent 5' and 3' untranslated sequences. A probe derived from the region downstream of the 3' homology fragment and upstream of the most distal HindIII site shown hybridizes to an 11.2 kb HindIII fragment in the GDF-8 gene and a 10.4 kb fragment in an homologously targeted gene. Abbreviations: H, HindIII; X, Xba I.

Figure 12b shows a Southern blot analysis of offspring derived from a mating of heterozygous mutant mice. The lanes are as follows: DNA prepared from wild type SV/J mice (lane 1), targeted embryonic stem cells (lane 2), F1 heterozygous mice (lanes 3 and 4), and offspring derived from a mating of these mice (lanes 5-13).

Figure 13 shows the muscle fiber size distribution in mutant and wild type littermates. Figure 13a shows the smallest cross-sectional fiber widths measured for wild type (n = 1761) and mutant (n = 1052) tibialis cranial. Figure 13b shows wild type (n = 900) and mutant (n = 900) gastrocnemius muscles, and fiber sizes were plotted as a percent of total fiber number. Standard deviations were 9 and 10 μ m, respectively, for wild type and mutant tibialis cranial is and 11 and 9 μ m, respectively, for wild type and mutant gastrocnemius muscles. Legend: o-o, wild type; _-_, mutant.

Figure 14a shows the nucleotide and deduced amino acid sequence for baboon GDF-8 (SEQ ID NO:18 and 19, respectively).

Figure 14b shows the nucleotide and deduced amino acid sequence for bovine GDF-8 (SEQ ID NO: 20 and 21, respectively).

Figure 14c shows the nucleotide and deduced amino acid sequence for chicken GDF-8 (SEQ ID NO:22 and 23, respectively).

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Figure 14d shows the nucleotide and deduced amino acid sequence for rat GDF-8 (SEQ ID NO:24 and 25, respectively).

Figure 14e shows the nucleotide and deduced amino acid sequence for turkey GDF-8 (SEQ ID NO:26 and 27, respectively).

- 5 Figure 14f shows the nucleotide and deduced amino acid sequence for porcine GDF-8 (SEQ ID NO:28 and 29, respectively).

Figure 14g shows the nucleotide and deduced amino acid sequence for ovine GDF-8 (SEQ ID NO:30 and 31, respectively).

- Figures 15a and 15b show an alignment between murine, rat, human, porcine, ovine,
10 baboon, bovine, chicken, and turkey GDF-8 amino acid sequences (SEQ ID NO:12, 25, 14, 29, 31, 19, 21, 23 and 27, respectively).

- Figure 16 shows the predicted amino acid sequences of murine and human GDF-11 aligned with murine (McPherron et al., 1997) and human (McPherron and Lee, 1997) myostatin (MSTN). Shaded boxes represent amino acid homology with the murine and
15 human GDF-11 sequences. Amino acids are numbered relative to the human GDF-11 sequence. The predicted proteolytic processing sites are located at amino acids 295-298.

- Figure 17 shows the construction of GDF-11 null mice by homologous targeting. a) is a map of the GDF-11 locus (top line) and targeting construct (second line). The black and stippled boxes represent coding sequences for the pro-and C-terminal regions,
20 respectively. The targeting construct contains a total of 11 kb of homology with the GDF-11 gene. A probe derived from the region upstream of the 3' homology fragment and downstream of the first EcoRI site shown hybridizes to a 6.5 kb EcoRI fragment in the GDF-11 gene and a 4.8 kb fragment in a homologously targeted gene. Abbreviations: X, XbaI; E, EcoRI. b) Geneomic Southern of DNA prepared from F1 heterozy-

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gous mutant mice (lanes 1 and 2) and offspring derived from a mating of these mice (lanes 3-12).

Figure 18 shows kidney abnormalities in GDF-11 knockout mice. Kidneys of newborn animals were examined and classified according to the number of normal sized or small
5 kidneys as shown at the top. Numbers in the table indicate number of animals falling into each classification according to genotype.

Figure 19 shows homeotic transformations in GDF-11 mutant mice. a) Newborn pups with missing (first and second from left) and normal looking tails. b-j) Skeleton preparations for newborn wild-type (b, e, h), heterozygous (c, f, i) and homozygous (d,
10 g, j) mutant mice. Whole skeleton preparations (b-d), vertebral columns (e-g), vertebrosteral ribs (h-j) showing transformations and defects in homozygous and heterozygous mutant mice. Numbers indicate thoracic segments.

Figure 20 is a table summarizing the anterior transformations in wild-type, heterozygous and homozygous GDF-11 mice.

15 DETAILED DESCRIPTION OF THE INVENTION

The present invention provides a growth and differentiation factor, GDF-8 and a polynucleotide sequence encoding GDF-8. GDF-8 is expressed at highest levels in muscle and at lower levels in adipose tissue.

The animals contemplated for use in the practice of the subject invention are those
20 animals generally regarded as useful for the processing of food stuffs, *i.e.* avian such as meat bred and egg laying chicken and turkey, ovine such as lamb, bovine such as beef cattle and milk cows, piscine and porcine. For purposes of the subject invention, these animals are referred to as "transgenic" when such animal has had a heterologous DNA sequence, or one or more additional DNA sequences normally endogenous to the animal
25 (collectively referred to herein as "transgenes") chromosomally integrated into the germ

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cells of the animal. The transgenic animal (including its progeny) will also have the transgene integrated into the chromosomes of somatic cells.

The TGF- β superfamily consists of multifunctional polypeptides that control proliferation, differentiation, and other functions in many cell types. Many of the peptides have
5 regulatory, both positive and negative, effects on other peptide growth factors. The structural homology between the GDF-8 protein of this invention and the members of the TGF- β family, indicates that GDF-8 is a new member of the family of growth and differentiation factors. Based on the known activities of many of the other members, it can be expected that GDF-8 will also possess biological activities that will make it useful
10 as a diagnostic and therapeutic reagent.

In particular, certain members of this superfamily have expression patterns or possess activities that relate to the function of the nervous system. For example, the inhibins and activins have been shown to be expressed in the brain (Meunier, *et al.*, *Proc. Natl. Acad. Sci., USA*, 85:247, 1988; Sawchenko, *et al.*, *Nature*, 334:615, 1988), and activin has been
15 shown to be capable of functioning as a nerve cell survival molecule (Schubert, *et al.*, *Nature*, 344:868, 1990). Another family member, namely, GDF-1, is nervous system-specific in its expression pattern (Lee, S.J., *Proc. Natl. Acad. Sci., USA*, 88:4250, 1991), and certain other family members, such as Vgr-1 (Lyons, *et al.*, *Proc. Natl. Acad. Sci., USA*, 86:4554, 1989; Jones, *et al.*, *Development*, 111:531, 1991), OP-1 (Ozkaynak,
20 *et al.*, *J. Biol. Chem.*, 267:25220, 1992), and BMP-4 (Jones, *et al.*, *Development*, 111:531, 1991), are also known to be expressed in the nervous system. Because it is known that skeletal muscle produces a factor or factors that promote the survival of motor neurons (Brown, *Trends Neurosci.*, 7:10, 1984), the expression of GDF-8 in muscle suggests that one activity of GDF-8 may be as a trophic factor for neurons. In this
25 regard, GDF-8 may have applications in the treatment of neurodegenerative diseases, such as amyotrophic lateral sclerosis or muscular dystrophy, or in maintaining cells or tissues in culture prior to transplantation.

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GDF-8 may also have applications in treating disease processes involving the musculoskeletal system, such as in musculodegenerative diseases, osteoporosis or in tissue repair due to trauma. In this regard, many other members of the TGF- β family are also important mediators of tissue repair. TGF- β has been shown to have marked effects on the formation of collagen and to cause a striking angiogenic response in the newborn mouse (Roberts, *et al.*, *Proc. Natl. Acad. Sci., USA* 83:4167, 1986). TGF- β has also been shown to inhibit the differentiation of myoblasts in culture (Massague, *et al.*, *Proc. Natl. Acad. Sci., USA* 83:8206, 1986). Moreover, because myoblast cells may be used as a vehicle for delivering genes to muscle for gene therapy, the properties of GDF-8 could be exploited for maintaining cells prior to transplantation or for enhancing the efficiency of the fusion. GDF-8 may also have applications in treating disease processes involving the kidney or in kidney repair due to trauma.

The expression of GDF-8 in adipose tissue also raises the possibility of applications for GDF-8 in the treatment of obesity or of disorders related to abnormal proliferation of adipocytes. In this regard, TGF- β has been shown to be a potent inhibitor of adipocyte differentiation *in vitro* (Ignotz and Massague, *Proc. Natl. Acad. Sci., USA* 82:8530, 1985).

Polypeptides, Polynucleotides, Vectors and Host Cells

The invention provides substantially pure GDF-8 polypeptide and isolated polynucleotides that encode GDF-8. The term "substantially pure" as used herein refers to GDF-8 which is substantially free of other proteins, lipids, carbohydrates or other materials with which it is naturally associated. One skilled in the art can purify GDF-8 using standard techniques for protein purification. The substantially pure polypeptide will yield a single major band on a non-reducing polyacrylamide gel. The purity of the GDF-8 polypeptide can also be determined by amino-terminal amino acid sequence analysis. GDF-8 polypeptide includes functional fragments of the polypeptide, as long as the activity of GDF-8 remains. Smaller peptides containing the biological activity of GDF-8 are included in the invention.

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The invention provides polynucleotides encoding the GDF-8 protein. These polynucleotides include DNA, cDNA and RNA sequences which encode GDF-8. It is understood that all polynucleotides encoding all or a portion of GDF-8 are also included herein, as long as they encode a polypeptide with GDF-8 activity. Such polynucleotides include
5 naturally occurring, synthetic, and intentionally manipulated polynucleotides. For example, GDF-8 polynucleotide may be subjected to site-directed mutagenesis. The polynucleotide sequence for GDF8 also includes antisense sequences. The polynucleotides of the invention include sequences that are degenerate as a result of the genetic code. There are 20 natural amino acids, most of which are specified by more than one
10 codon. Therefore, all degenerate nucleotide sequences are included in the invention as long as the amino acid sequence of GDF-8 polypeptide encoded by the nucleotide sequence is functionally unchanged.

Specifically disclosed herein is a genomic DNA sequence containing a portion of the GDF-8 gene. The sequence contains an open reading frame corresponding to the
15 predicted C-terminal region of the GDF-8 precursor protein. The encoded polypeptide is predicted to contain two potential proteolytic processing sites (KR and RR). Cleavage of the precursor at the downstream site would generate a mature biologically active C-terminal fragment of 109 and 103 amino acids for murine and human species, respectively, with a predicted molecular weight of approximately 12,400. Also disclosed
20 are full length murine and human GDF-8 cDNA sequences. The murine pre-pro-GDF-8 protein is 376 amino acids in length, which is encoded by a 2676 base pair nucleotide sequence, beginning at nucleotide 104 and extending to a TGA stop codon at nucleotide 1232. The human GDF-8 protein is 375 amino acids and is encoded by a 2743 base pair sequence, with the open reading frame beginning at nucleotide 59 and extending to
25 nucleotide 1184. GDF-8 is also capable of forming dimers, or heterodimers, with an expected molecular weight of approximately 23-30KD (see Example 4). For example, GDF-8 may form heterodimers with other family members, such as GDF-11.

Also provided herein are the biologically active C-terminal fragments of chicken (Figure 2c) and rat (Figure 2d) GDF-8. The full length nucleotide and deduced amino acid

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sequences for baboon, bovine, chicken, rat, ovine, porcine, and turkey are shown in Figures 14a-g and human and murine are shown in Figure 5. As shown in Figure 3b, alignment of the amino acid sequences of human, murine, rat and chicken GDF-8 indicate that the sequences are 100% identical in the C-terminal biologically active
5 fragment. Figure 15 a and 15b also show the alignment of GDF-8 amino acid sequences for murine, rat, human, baboon, porcine, ovine, bovine, chicken and turkey. Given the extensive conservation of amino acid sequences between species, it would now be routine for one of skill in the art to obtain the GDF-8 nucleic acid and amino acid sequence for GDF-8 from any species, including those provided herein, as well as
10 piscine, for example.

The C-terminal region of GDF-8 following the putative proteolytic processing site shows significant homology to the known members of the TGF- β superfamily. The GDF-8 sequence contains most of the residues that are highly conserved in other family members and in other species (see FIGURES 3a and 3b and 15 a and 15b). Like the
15 TGF- β s and inhibin β s, GDF-8 contains an extra pair of cysteine residues in addition to the 7 cysteines found in virtually all other family members. Among the known family members, GDF-8 is most homologous to Vgr-1 (45% sequence identity) (see FIGURE 4).

Minor modifications of the recombinant GDF-8 primary amino acid sequence may result
20 in proteins which have substantially equivalent activity as compared to the GDF-8 polypeptide described herein. Such modifications may be deliberate, as by site-directed mutagenesis, or may be spontaneous. All of the polypeptides produced by these modifications are included herein as long as the biological activity of GDF-8 still exists. Further, deletion of one or more amino acids can also result in a modification of the
25 structure of the resultant molecule without significantly altering its biological activity. This can lead to the development of a smaller active molecule which would have broader utility. For example, one can remove amino or carboxy terminal amino acids which are not required for GDF-8 biological activity.

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The nucleotide sequence encoding the GDF-8 polypeptide of the invention includes the disclosed sequence and conservative variations thereof. The term "conservative variation" as used herein denotes the replacement of an amino acid residue by another, biologically similar residue. Examples of conservative variations include the substitution
5 of one hydrophobic residue such as isoleucine, valine, leucine or methionine for another, or the substitution of one polar residue for another, such as the substitution of arginine for lysine, glutamic for aspartic acid, or glutamine for asparagine, and the like. The term "conservative variation" also includes the use of a substituted amino acid in place of an unsubstituted parent amino acid provided that antibodies raised to the substituted
10 polypeptide also immunoreact with the unsubstituted polypeptide.

DNA sequences of the invention can be obtained by several methods. For example, the DNA can be isolated using hybridization techniques which are well known in the art. These include, but are not limited to: 1) hybridization of genomic or cDNA libraries with probes to detect homologous nucleotide sequences, 2) polymerase chain reaction (PCR)
15 on genomic DNA or cDNA using primers capable of annealing to the DNA sequence of interest, and 3) antibody screening of expression libraries to detect cloned DNA fragments with shared structural features.

Preferably the GDF-8 polynucleotide of the invention is derived from a mammalian organism, and most preferably from mouse, rat, cow, pig, or human. GDF-8 polynucleo-
20 tides from chicken, turkey, fish and other species are also included herein. Screening procedures which rely on nucleic acid hybridization make it possible to isolate any gene sequence from any organism, provided the appropriate probe is available. Given the extensive nucleotide and amino acid homology between species, it would be routine for one of skill in the art to obtain polynucleotides encoding GDF-8 from any species. -
25 Oligonucleotide probes, which correspond to a part of the sequence encoding the protein in question, can be synthesized chemically. This requires that short, oligopeptide stretches of amino acid sequence must be known. The DNA sequence encoding the protein can be deduced from the genetic code, however, the degeneracy of the code must be taken into account. It is possible to perform a mixed addition reaction when the

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sequence is degenerate. This includes a heterogeneous mixture of denatured double-stranded DNA. For such screening, hybridization is preferably performed on either single-stranded DNA or denatured double-stranded DNA. Hybridization is particularly useful in the detection of cDNA clones derived from sources where an extremely low amount of mRNA sequences relating to the polypeptide of interest are present. In other words, by using stringent hybridization conditions directed to avoid non-specific binding, it is possible, for example, to allow the autoradiographic visualization of a specific cDNA clone by the hybridization of the target DNA to that single probe in the mixture which is its complete complement (Wallace, *et al.*, *Nucl. Acid Res.* 9:879, 1981).

- 10 The development of specific DNA sequences encoding GDF-8 can also be obtained by:
1) isolation of double-stranded DNA sequences from the genomic DNA; 2) chemical manufacture of a DNA sequence to provide the necessary codons for the polypeptide of interest; and 3) *in vitro* synthesis of a doublestranded DNA sequence by reverse transcription of mRNA isolated from a eukaryotic donor cell. In the latter case, a double-stranded DNA complement of mRNA is eventually formed which is generally referred to as cDNA.

Of the three above-noted methods for developing specific DNA sequences for use in recombinant procedures, the isolation of genomic DNA isolates is the least common. This is especially true when it is desirable to obtain the microbial expression of mammalian polypeptides due to the presence of introns.

The synthesis of DNA sequences is frequently the method of choice when the entire sequence of amino acid residues of the desired polypeptide product is known. When the entire sequence of amino acid residues of the desired polypeptide is not known, the direct synthesis of DNA sequences is not possible and the method of choice is the synthesis of cDNA sequences. Among the standard procedures for isolating cDNA sequences of interest is the formation of plasmid- or phage-carrying cDNA libraries which are derived from reverse transcription of mRNA which is abundant in donor cells that have a high level of genetic expression. When used in combination with polymerase chain reaction

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technology, even rare expression products can be cloned. In those cases where significant portions of the amino acid sequence of the polypeptide are known, the production of labeled single or double-stranded DNA or RNA probe sequences duplicating a sequence putatively present in the target cDNA may be employed in DNA/DNA hybridization
5 procedures which are carried out on cloned copies of the cDNA which have been denatured into a single-stranded form (Jay, *et al.*, *Nucl. Acid Res.*, 11:2325, 1983).

A cDNA expression library, such as lambda gt11, can be screened indirectly for GDF-8 peptides having at least one epitope, using antibodies specific for GDF-8. Such antibodies can be either polyclonally or monoclonally derived and used to detect
10 expression product indicative of the presence of GDF-8 cDNA.

In nucleic acid hybridization reactions, the conditions used to achieve a particular level of stringency will vary, depending on the nature of the nucleic acids being hybridized. For example, the length, degree of complementarity, nucleotide sequence composition (*e.g.*, GC v. AT content), and nucleic acid type (*e.g.*, RNA v. DNA) of the hybridizing
15 regions of the nucleic acids can be considered in selecting hybridization conditions. An additional consideration is whether one of the nucleic acids is immobilized, for example, on a filter.

An example of progressively higher stringency conditions is as follows: 2 x SSC/0.1% SDS at about room temperature (hybridization conditions); 0.2 x SSC/0.1% SDS at about
20 room temperature (low stringency conditions); 0.2 x SSC/0.1% SDS at about 42°C (moderate stringency conditions); and 0.1 x SSC at about 68°C (high stringency conditions). Washing can be carried out using only one of these conditions, *e.g.*, high stringency conditions, or each of the conditions can be used, *e.g.*, for 10-15 minutes each, in the order listed above, repeating any or all of the steps listed. However, as mentioned
25 above, optimal conditions will vary, depending on the particular hybridization reaction involved, and can be determined empirically.

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DNA sequences encoding GDF-8 can be expressed *in vitro* by DNA transfer into a suitable host cell. "Host cells" are cells in which a vector can be propagated and its DNA expressed. The term also includes any progeny of the subject host cell. It is understood that all progeny may not be identical to the parental cell since there may be mutations
5 that occur during replication. However, such progeny are included when the term "host cell" is used. Methods of stable transfer, meaning that the foreign DNA is continuously maintained in the host, are known in the art.

In the present invention, the GDF-8 polynucleotide sequences may be inserted into a recombinant expression vector. The term "recombinant expression vector" refers to a
10 plasmid, virus or other vehicle known in the art that has been manipulated by insertion or incorporation of the GDF-8 genetic sequences. Such expression vectors contain a promoter sequence which facilitates the efficient transcription of the inserted genetic sequence of the host. The expression vector typically contains an origin of replication, a promoter, as well as specific genes which allow phenotypic selection of the trans-
15 formed cells. Vectors suitable for use in the present invention include, but are not limited to the T7-based expression vector for expression in bacteria (Rosenberg, *et al.*, *Gene*, 56:125, 1987), the pMSXND expression vector for expression in mammalian cells (Lee and Nathans, *J. Biol. Chem.*, 263:3521, 1988) and baculovirus-derived vectors for expression in insect cells. The DNA segment can be present in the vector operably linked
20 to regulatory elements, for example, a promoter (*e.g.*, T7, metallothionein 1, or polyhedrin promoters).

Polynucleotide sequences encoding GDF-8 can be expressed in either prokaryotes or eukaryotes. Hosts can include microbial, yeast, insect and mammalian organisms. Methods of expressing DNA sequences having eukaryotic or viral sequences in
25 prokaryotes are well known in the art. Biologically functional viral and plasmid DNA vectors capable of expression and replication in a host are known in the art. Such vectors are used to incorporate DNA sequences of the invention. Preferably, the mature C-terminal region of GDF-8 is expressed from a cDNA clone containing the entire coding sequence of GDF-8. Alternatively, the C-terminal portion of GDF-8 can be

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expressed as a fusion protein with the pro- region of another member of the TGF- β family or co-expressed with another pro-region (see for example, Hammonds, *et al.*, *Molec. Endocrin.*, 5:149, 1991; Gray, A., and Mason, A., *Science*, 247:1328, 1990).

Transformation of a host cell with recombinant DNA may be carried out by conventional techniques as are well known to those skilled in the art. Where the host is prokaryotic, such as *E. coli*, competent cells which are capable of DNA uptake can be prepared from cells harvested after exponential growth phase and subsequently treated by the CaCl_2 method using procedures well known in the art. Alternatively, MgCl_2 or RbCl can be used. Transformation can also be performed after forming a protoplast of the host cell if desired.

When the host is a eukaryote, such methods of transfection of DNA as calcium phosphate co-precipitates, conventional mechanical procedures such as microinjection, electroporation, insertion of a plasmid encased in liposomes, or virus vectors may be used. Eukaryotic cells can also be cotransformed with DNA sequences encoding the GDF-8 of the invention, and a second foreign DNA molecule encoding a selectable phenotype, such as the herpes simplex thymidine kinase gene. Another method is to use a eukaryotic viral vector, such as simian virus 40 (SV40) or bovine papilloma virus, to transiently infect or transform eukaryotic cells and express the protein. (see for example, *Eukaryotic Viral Vectors*, Cold Spring Harbor Laboratory, Gluzman ed., 1982).

Isolation and purification of microbial expressed polypeptide, or fragments thereof, provided by the invention, may be carried out by conventional means including preparative chromatography and immunological separations involving monoclonal or polyclonal antibodies.

GDF-8 Antibodies and Methods of Use

The invention includes antibodies immunoreactive with GDF-8 polypeptide or functional fragments thereof. Antibody which consists essentially of pooled monoclonal antibodies with different epitopic specificities, as well as distinct monoclonal antibody preparations

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are provided. Monoclonal antibodies are made from antigen containing fragments of the protein by methods well known to those skilled in the art (Kohler, *et al.*, *Nature*, 256:495, 1975). The term antibody as used in this invention is meant to include intact molecules as well as fragments thereof, such as Fab and F(ab')₂, Fv and SCA fragments
5 which are capable of binding an epitopic determinant on GDF-8.

(1) An Fab fragment consists of a monovalent antigen-binding fragment of an antibody molecule, and can be produced by digestion of a whole antibody molecule with the enzyme papain, to yield a fragment consisting of an intact light chain and a portion of a heavy chain.

10 (2) An Fab' fragment of an antibody molecule can be obtained by treating a whole antibody molecule with pepsin, followed by reduction, to yield a molecule consisting of an intact light chain and a portion of a heavy chain. Two Fab' fragments are obtained per antibody molecule treated in this manner.

(3) An (Fab')₂ fragment of an antibody can be obtained by treating a whole antibody
15 molecule with the enzyme pepsin, without subsequent reduction. A (Fab')₂ fragment is a dimer of two Fab' fragments, held together by two disulfide bonds.

(4) An Fv fragment is defined as a genetically engineered fragment containing the variable region of a light chain and the variable region of a heavy chain expressed as two chains.

20 (5) A single chain antibody ("SCA") is a genetically engineered single chain molecule containing the variable region of a light chain and the variable region of a heavy chain, linked by a suitable, flexible polypeptide linker.

As used in this invention, the term "epitope" refers to an antigenic determinant on an antigen, such as a GDF-8 polypeptide, to which the paratope of an antibody, such as an
25 GDF-8-specific antibody, binds. Antigenic determinants usually consist of chemically

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active surface groupings of molecules, such as amino acids or sugar side chains, and can have specific three-dimensional structural characteristics, as well as specific charge characteristics.

As is mentioned above, antigens that can be used in producing GDF-8-specific antibodies
5 include GDF-8 polypeptides or GDF-8 polypeptide fragments. The polypeptide or peptide used to immunize an animal can be obtained by standard recombinant, chemical synthetic, or purification methods. As is well known in the art, in order to increase immunogenicity, an antigen can be conjugated to a carrier protein. Commonly used carriers include keyhole limpet hemocyanin (KLH), thyroglobulin, bovine serum albumin
10 (BSA), and tetanus toxoid. The coupled peptide is then used to immunize the animal (*e.g.*, a mouse, a rat, or a rabbit). In addition to such carriers, well known adjuvants can be administered with the antigen to facilitate induction of a strong immune response.

The term "cell-proliferative disorder" denotes malignant as well as non-malignant cell populations which often appear to differ from the surrounding tissue both morphologi-
15 cally and genotypically. Malignant cells (*i.e.* cancer) develop as a result of a multistep process. The GDF-8 polynucleotide that is an antisense molecule or that encodes a dominant negative GDF-8 is useful in treating malignancies of the various organ systems, particularly, for example, cells in muscle, bone, kidney or adipose tissue. Essentially, any disorder which is etiologically linked to altered expression of GDF-8 could be considered
20 susceptible to treatment with a GDF-8 agent (*e.g.*, a suppressing or enhancing agent). One such disorder is a malignant cell proliferative disorder, for example.

The invention provides a method for detecting a cell proliferative disorder of muscle, bone, kidney or adipose tissue which comprises contacting an anti-GDF-8 antibody with
25 a cell suspected of having a GDF-8 associated disorder and detecting binding to the antibody. The antibody reactive with GDF-8 is labeled with a compound which allows detection of binding to GDF-8. For purposes of the invention, an antibody specific for GDF-8 polypeptide may be used to detect the level of GDF-8 in biological fluids and tissues. Any specimen containing a detectable amount of antigen can be used. Preferred

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samples of this invention include muscle, bone or kidney tissue. The level of GDF-8 in the suspect cell can be compared with the level in a normal cell to determine whether the subject has a GDF-8-associated cell proliferative disorder. Such methods of detection are also useful using nucleic acid hybridization to detect the level of GDF-8 mRNA in
5 a sample or to detect an altered GDF-8 gene. Preferably the subject is human.

The antibodies of the invention can be used in any subject in which it is desirable to administer *in vitro* or *in vivo* immunodiagnosis or immunotherapy. The antibodies of the invention are suited for use, for example, in immunoassays in which they can be utilized in liquid phase or bound to a solid phase carrier. In addition, the antibodies in these
10 immunoassays can be detectably labeled in various ways. Examples of types of immunoassays which can utilize antibodies of the invention are competitive and non-competitive immunoassays in either a direct or indirect format. Examples of such immunoassays are the radioimmunoassay (RIA) and the sandwich (immunometric) assay. Detection of the antigens using the antibodies of the invention can be done utilizing
15 immunoassays which are run in either the forward, reverse, or simultaneous modes, including immunohistochemical assays on physiological samples. Those of skill in the art will know, or can readily discern, other immunoassay formats without undue experimentation.

The antibodies of the invention can be bound to many different carriers and used to
20 detect the presence of an antigen comprising the polypeptide of the invention. Examples of well-known carriers include glass, polystyrene, polypropylene, polyethylene, dextran, nylon, amylases, natural and modified celluloses, polyacrylamides, agaroses and magnetite. The nature of the carrier can be either soluble or insoluble for purposes of the invention. Those skilled in the art will know of other suitable carriers for binding
25 antibodies, or will be able to ascertain such, using routine experimentation.

There are many different labels and methods of labeling known to those of ordinary skill in the art. Examples of the types of labels which can be used in the present invention include enzymes, radioisotopes, fluorescent compounds, colloidal metals,

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chemiluminescent compounds, phosphorescent compounds, and bioluminescent compounds. Those of ordinary skill in the art will know of other suitable labels for binding to the antibody, or will be able to ascertain such, using routine experimentation.

Another technique which may also result in greater sensitivity consists of coupling the
5 antibodies to low molecular weight haptens. These haptens can then be specifically detected by means of a second reaction. For example, it is common to use such haptens as biotin, which reacts with avidin, or dinitrophenyl, puridoxal, and fluorescein, which can react with specific antihapten antibodies.

In using the monoclonal antibodies of the invention for the *in vivo* detection of antigen,
10 the detectably labeled antibody is given a dose which is diagnostically effective. The term "diagnostically effective" means that the amount of detectably labeled monoclonal antibody is administered in sufficient quantity to enable detection of the site having the antigen comprising a polypeptide of the invention for which the monoclonal antibodies are specific.

15 The concentration of detectably labeled monoclonal antibody which is administered should be sufficient such that the binding to those cells having the polypeptide is detectable compared to the background. Further, it is desirable that the detectably labeled monoclonal antibody be rapidly cleared from the circulatory system in order to give the best target-to-background signal ratio.

20 As a rule, the dosage of detectably labeled monoclonal antibody for *in vivo* diagnosis will vary depending on such factors as age, sex, and extent of disease of the individual. Such dosages may vary, for example, depending on whether multiple injections are given, antigenic burden, and other factors known to those of skill in the art.

For *in vivo* diagnostic imaging, the type of detection instrument available is a major
25 factor in selecting a given radioisotope. The radioisotope chosen must have a type of decay which is detectable for a given type of instrument. Still another important factor

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in selecting a radioisotope for *in vivo* diagnosis is that deleterious radiation with respect to the host is minimized. Ideally, a radioisotope used for *in vivo* imaging will lack a particle emission, but produce a large number of photons in the 140-250 keV range, which may readily be detected by conventional gamma cameras.

- 5 For *in vivo* diagnosis radioisotopes may be bound to immunoglobulin either directly or indirectly by using an intermediate functional group. intermediate functional groups which often are used to bind radioisotopes which exist as metallic ions to immunoglobulins are the bifunctional chelating agents such as diethylenetriaminepentaacetic acid (DTPA) and ethylenediaminetetraacetic acid (EDTA)
- 10 and similar molecules. Typical examples of metallic ions which can be bound to the monoclonal antibodies of the invention are ^{111}In , ^{97}Ru , ^{67}Ga , ^{68}Ga , ^{72}As , ^{89}Zr and ^{201}Tl .

The monoclonal antibodies of the invention can also be labeled with a paramagnetic isotope for purposes of *in vivo* diagnosis, as in magnetic resonance imaging (MRI) or electron spin resonance (ESR). In general, any conventional method for visualizing

15 diagnostic imaging can be utilized. Usually gamma and positron emitting radioisotopes are used for camera imaging and paramagnetic isotopes for MRI. Elements which are particularly useful in such techniques include ^{157}Gd , ^{55}Mn , ^{162}Dy , ^{52}Cr , and ^{56}Fe .

The monoclonal antibodies of the invention can be used *in vitro* and *in vivo* to monitor the course of amelioration of a GDF-8-associated disease in a subject. Thus, for example,

20 by measuring the increase or decrease in the number of cells expressing antigen comprising a polypeptide of the invention or changes in the concentration of such antigen present in various body fluids, it would be possible to determine whether a particular therapeutic regimen aimed at ameliorating the GDF-8-associated disease is effective. The term "ameliorate" denotes a lessening of the detrimental effect of the GDF-8-associated

25 disease in the subject receiving therapy.

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Additional Methods of Treatment and Diagnosis

The present invention identifies a nucleotide sequence that can be expressed in an altered manner as compared to expression in a normal cell, therefore it is possible to design appropriate therapeutic or diagnostic techniques directed to this sequence. Treatment includes administration of a reagent which modulates activity. The term "modulate" envisions the suppression or expression of GDF-8 when it is over-expressed, or augmentation of GDF-8 expression when it is underexpressed. When a muscle or bone-associated disorder is associated with GDF-8 overexpression, such suppressive reagents as antisense GDF-8 polynucleotide sequence, dominant negative sequences or GDF-8 binding antibody can be introduced into a cell. In addition, an anti-idiotypic antibody which binds to a monoclonal antibody which binds GDF-8 of the invention, or an epitope thereof, may also be used in the therapeutic method of the invention. Alternatively, when a cell proliferative disorder is associated with underexpression or expression of a mutant GDF-8 polypeptide, a sense polynucleotide sequence (the DNA coding strand) or GDF-8 polypeptide can be introduced into the cell. Such muscle or bone-associated disorders include cancer, muscular dystrophy, spinal cord injury, traumatic injury, congestive obstructive pulmonary disease (COPD), AIDS or cachecia. In addition, the method of the invention can be used in the treatment of obesity or of disorders related to abnormal proliferation of adipocytes. One of skill in the art can determine whether or not a particular therapeutic course of treatment is successful by several methods described herein (e.g., muscle fiber analysis or biopsy; determination of fat content). The present examples demonstrate that the methods of the invention are useful for decreasing fat content, and therefore would be useful in the treatment of obesity and related disorders (e.g., diabetes). Neurodegenerative disorders are also envisioned as treated by the method of the invention.

Thus, where a cell-proliferative disorder is associated with the expression of GDF-8, nucleic acid sequences that interfere with GDF-8 expression at the translational level can be used. This approach utilizes, for example, antisense nucleic acid and ribozymes to block translation of a specific GDF-8 mRNA, either by masking that mRNA with an antisense nucleic acid or by cleaving it with a ribozyme. Such disorders include

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neurodegenerative diseases, for example. In addition, dominant-negative GDF-8 mutants would be useful to actively interfere with function of "normal" GDF-8.

Antisense nucleic acids are DNA or RNA molecules that are complementary to at least a portion of a specific mRNA molecule (Weintraub, *Scientific American*, 262:40, 1990).

- 5 In the cell, the antisense nucleic acids hybridize to the corresponding mRNA, forming a double-stranded molecule. The antisense nucleic acids interfere with the translation of the mRNA, since the cell will not translate a mRNA that is double-stranded.

- Antisense oligomers of about 15 nucleotides are preferred, since they are easily synthesized and are less likely to cause problems than larger molecules when introduced
10 into the target GDF-8-producing cell. The use of antisense methods to inhibit the *in vitro* translation of genes is well known in the art (Marcus-Sakura, *Anal. Biochem.*, 172:289, 1988).

- Ribozymes are RNA molecules possessing the ability to specifically cleave other single-stranded RNA in a manner analogous to DNA restriction endonucleases. Through
15 the modification of nucleotide sequences which encode these RNAs, it is possible to engineer molecules that recognize specific nucleotide sequences in an RNA molecule and cleave it (Cech, *J. Amer. Med. Assn.*, 260:3030, 1988). A major advantage of this approach is that, because they are sequence-specific, only mRNAs with particular sequences are inactivated.

- 20 There are two basic types of ribozymes namely, tetrahymena-type (Hasselhoff, *Nature*, 334:585, 1988) and "hammerhead"-type. Tetrahymena-type ribozymes recognize sequences which are four bases in length, while "hammerhead"-type ribozymes recognize base sequences 11-18 bases in length. The longer the recognition sequence, the greater the likelihood that the sequence will occur exclusively in the target mRNA species.
25 Consequently, hammerhead-type ribozymes are preferable to tetrahymena-type ribozymes for inactivating a specific mRNA species and 18-based recognition sequences are preferable to shorter recognition sequences.

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In another embodiment of the present invention, a nucleotide sequence encoding a GDF-8 dominant negative protein is provided. For example, a genetic construct that contain such a dominant negative encoding gene may be operably linked to a promoter, such as a tissue-specific promoter. For example, a skeletal muscle specific promoter (*e.g.*,
5 human skeletal muscle α -actin promoter) or developmentally specific promoter (*e.g.*, MyHC 3, which is restricted in skeletal muscle to the embryonic period of development, or an inducible promoter (*e.g.*, the orphan nuclear receptor TIS1).

Such constructs are useful in methods of modulating a subject's skeletal mass. For example, a method include transforming an organism, tissue, organ or cell with a genetic
10 construct encoding a dominant negative GDF-8 protein and suitable promoter in operable linkage and expressing the dominant negative encoding GDF-8 gene, thereby modulating muscle and/or bone mass by interfering with wild-type GDF-8 activity.

GDF-8 most likely forms dimers, homodimers or heterodimers and may even form heterodimers with other GDF family members, such as GDF-11 (*see* Example 4). Hence,
15 while not wanting to be bound by a particular theory, the dominant negative effect described herein may involve the formation of non-functional homodimers or heterodimers of dominant negative and wild-type GDF-8 monomers. More specifically, it is possible that any non-functional homodimer or any heterodimer formed by the dimerization of wild-type and/or dominant negative GDF-8 monomers produces a
20 dominant effect by: 1) being synthesized but not processed or secreted; 2) inhibiting the secretion of wild type GDF-8; 3) preventing normal proteolytic cleavage of the preprotein thereby producing a nonfunctional GDF-8 molecule; 4) altering the affinity of the non-functional dimer (*e.g.*, homodimeric or heterodimeric GDF-8) to a receptor or generating an antagonistic form of GDF-8 that binds a receptor without activating it;
25 or 5) inhibiting the intracellular processing or secretion of GDF-8 related or TGF- β family proteins.

Non-functional GDF-8 can function to inhibit the growth regulating actions of GDF-8 on muscle and bone cells that include a dominant negative GDF-8 gene. Deletion or

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missense dominant negative forms of GDF-8 that retain the ability to form dimers with wild-type GDF-8 protein but do not function as wild-type GDF-8 proteins may be used to inhibit the biological activity of endogenous wild-type GDF-8. For example, in one embodiment, the proteolytic processing site of GDF-8 may be altered (*e.g.*, deleted) 5 resulting in a GDF-8 molecule able to undergo subsequent dimerization with endogenous wild-type GDF-8 but unable to undergo further processing into a mature GDF-8 form. Alternatively, a non-functional GDF-8 can function as a monomeric species to inhibit the growth regulating actions of GDF-8 on muscle or bone cells.

Any genetic recombinant method in the art may be used, for example, recombinant 10 viruses may be engineered to express a dominant negative form of GDF-8 which may be used to inhibit the activity of wild-type GDF-8. Such viruses may be used therapeutically for treatment of diseases resulting from aberrant over-expression or activity of GDF-8 protein, such as in denervation hypertrophy or as a means of controlling GDF-8 expression when treating disease conditions involving the musculoskeletal system, such 15 as in musculodegenerative diseases, osteoporosis or in tissue repair due to trauma or in modulating GDF-8 expression in animal husbandry (*e.g.*, transgenic animals for agricultural purposes).

The invention provides a method for treating a muscle, bone, kidney (chronic or acute) or adipose tissue disorder in a subject. The method includes administering a therapeuti- 20 cally effective amount of a GDF-8 agent to the subject, thereby inhibiting abnormal growth of muscle, bone, kidney or adipose tissue. The GDF-8 agent may include a GDF-8 antisense molecule or a dominant negative polypeptide, for example. A "therapeutically effective amount" of a GDF-8 agent is that amount that ameliorates symptoms of the disorder or inhibits GDF-8 induced growth of muscle or bone, for example, as 25 compared with a normal subject.

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Gene Therapy

The present invention also provides gene therapy for the treatment of cell proliferative or immunologic disorders which are mediated by GDF-8 protein. Such therapy would achieve its therapeutic effect by introduction of the GDF-8 antisense or dominant
5 negative encoding polynucleotide into cells having the proliferative disorder. Delivery of antisense or dominant negative GDF-8 polynucleotide can be achieved using a recombinant expression vector such as a chimeric virus or a colloidal dispersion system. Especially preferred for therapeutic delivery of antisense or dominant negative sequences is the use of targeted liposomes. In contrast, when it is desirable to enhance GDF-8
10 production, a "sense" GDF-8 polynucleotide or functional equivalent (*e.g.*, the C-term active region) is introduced into the appropriate cell(s).

Various viral vectors which can be utilized for gene therapy as taught herein include adenovirus, herpes virus, vaccinia, or, preferably, an RNA virus such as a retrovirus. Preferably, the retroviral vector is a derivative of a murine or avian retrovirus. Examples
15 of retroviral vectors in which a single foreign gene can be inserted include, but are not limited to: Moloney murine leukemia virus (MoMuLV), Harvey murine sarcoma virus (HaMuSV), murine mammary tumor virus (MuMTV), and Rous Sarcoma Virus (RSV). A number of additional retroviral vectors can incorporate multiple genes. All of these vectors can transfer or incorporate a gene for a selectable marker so that transduced cells
20 can be identified and generated. By inserting a GDF-8 sequence of interest into the viral vector, along with another gene which encodes the ligand for a receptor on a specific target cell, for example, the vector is now target specific. Retroviral vectors can be made target specific by attaching, for example, a sugar, a glycolipid, or a protein. Preferred targeting is accomplished by using an antibody to target the retroviral vector. Those of
25 skill in the art will know of, or can readily ascertain without undue experimentation, specific polynucleotide sequences which can be inserted into the retroviral genome or attached to a viral envelope to allow target specific delivery of the retroviral vector containing the GDF-8 antisense polynucleotide.

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Since recombinant retroviruses are defective, they require assistance in order to produce infectious vector particles. This assistance can be provided, for example, by using helper cell lines that contain plasmids encoding all of the structural genes of the retrovirus under the control of regulatory sequences within the LTR. These plasmids are missing a nucleotide sequence which enables the packaging mechanism to recognize an RNA transcript for encapsulation. Helper cell lines which have deletions of the packaging signal include, but are not limited to ψ 2, PA317 and PA12, for example. These cell lines produce empty virions, since no genome is packaged. If a retroviral vector is introduced into such cells in which the packaging signal is intact, but the structural genes are replaced by other genes of interest, the vector can be packaged and vector virion produced.

Alternatively, NIH 3T3 or other tissue culture cells can be directly transfected with plasmids encoding the retroviral structural genes gag, pol and env, by conventional calcium phosphate transfection. These cells are then transfected with the vector plasmid containing the genes of interest. The resulting cells release the retroviral vector into the culture medium.

Another targeted delivery system for GDF-8 polynucleotides is a colloidal dispersion system. Colloidal dispersion systems include macromolecule complexes, nanocapsules, microspheres, beads, and lipid-based systems including oil-in-water emulsions, micelles, mixed micelles, and liposomes. The preferred colloidal system of this invention is a liposome. Liposomes are artificial membrane vesicles which are useful as delivery vehicles *in vitro* and *in vivo*. It has been shown that large unilamellar vesicles (LUV), which range in size from 0.2-4.0 μ m can encapsulate a substantial percentage of an aqueous buffer containing large macromolecules. RNA, DNA and intact virions can be encapsulated within the aqueous interior and be delivered to cells in a biologically active form (Fraley, *et al.*, *Trends Biochem. Sci.*, 6:77, 1981). In addition to mammalian cells, liposomes have been used for delivery of polynucleotides in plant, yeast and bacterial cells. In order for a liposome to be an efficient gene transfer vehicle, the following characteristics should be present: (1) encapsulation of the genes of interest at high

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efficiency while not compromising their biological activity; (2) preferential and substantial binding to a target cell in comparison to non-target cells; (3) delivery of the aqueous contents of the vesicle to the target cell cytoplasm at high efficiency; and (4) accurate and effective expression of genetic information (Manning, *et al.*, *Biotechniques*,
5 6:682, 1988).

The composition of the liposome is usually a combination of phospholipids, particularly high-phase-transition-temperature phospholipids, usually in combination with steroids, especially cholesterol. Other phospholipids or other lipids may also be used. The physical characteristics of liposomes depend on pH, ionic strength, and the presence of divalent
10 cations.

Examples of lipids useful in liposome production include phosphatidyl compounds, such as phosphatidylglycerol, phosphatidylcholine, phosphatidylserine, phosphatidylethanolamine, sphingolipids, cerebroside, and gangliosides. Particularly useful are diacylphosphatidylglycerols, where the lipid moiety contains from 14-18
15 carbon atoms, particularly from 16-18 carbon atoms, and is saturated. Illustrative phospholipids include egg phosphatidylcholine, dipalmitoylphosphatidylcholine and distearoylphosphatidylcholine.

The targeting of liposomes can be classified based on anatomical and mechanistic factors. Anatomical classification is based on the level of selectivity, for example,
20 organ-specific, cell-specific, and organelle-specific. Mechanistic targeting can be distinguished based upon whether it is passive or active. Passive targeting utilizes the natural tendency of liposomes to distribute to cells of the reticulo-endothelial system (RES) in organs which contain sinusoidal capillaries. Active targeting, on the other hand, involves alteration of the liposome by coupling the liposome to a specific ligand such as
25 a monoclonal antibody, sugar, glycolipid, or protein, or by changing the composition or size of the liposome in order to achieve targeting to organs and cell types other than the naturally occurring sites of localization.

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The surface of the targeted delivery system may be modified in a variety of ways. In the case of a liposomal targeted delivery system, lipid groups can be incorporated into the lipid bilayer of the liposome in order to maintain the targeting ligand in stable association with the liposomal bilayer. Various linking groups can be used for joining the lipid chains to the targeting ligand.

Due to the expression of GDF-8 in muscle, bone, kidney and adipose tissue, there are a variety of applications using the polypeptide, polynucleotide, and antibodies of the invention, related to these tissues. Such applications include treatment of cell proliferative disorders involving these and other tissues, such as neural tissue. In addition, GDF-8 may be useful in various gene therapy procedures. In embodiments where GDF-8 polypeptide is administered to a subject, the dosage range is about 0.1 ug/kg to 100 mg/kg; more preferably from about 1 ug/kg to 75 mg/kg and most preferably from about 10 mg/kg to 50 mg/kg.

Chromosomal Location of GDF-8

The data in Example 6 shows that the human GDF-8 gene is located on chromosome 2. By comparing the chromosomal location of GDF-8 with the map positions of various human disorders, it should be possible to determine whether mutations in the GDF-8 gene are involved in the etiology of human diseases. For example, an autosomal recessive form of juvenile amyotrophic lateral sclerosis has been shown to map to chromosome 2 (Hentati, *et al.*, *Neurology*, 42 [Suppl.3]:201, 1992). More precise mapping of GDF-8 and analysis of DNA from these patients may indicate that GDF-8 is, in fact, the gene affected in this disease. In addition, GDF-8 is useful for distinguishing chromosome 2 from other chromosomes.

Transgenic Animals and Methods of Making the same

Various methods to make the transgenic animals of the subject invention can be employed. Generally speaking, three such methods may be employed. In one such method, an embryo at the pronuclear stage (a "one cell embryo") is harvested from a female and the transgene is microinjected into the embryo, in which case the transgene

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will be chromosomally integrated into both the germ cells and somatic cells of the resulting mature animal. In another such method, embryonic stem cells are isolated and the transgene incorporated therein by electroporation, plasmid transfection or microinjection, followed by reintroduction of the stem cells into the embryo where they
5 colonize and contribute to the germ line. Methods for microinjection of mammalian species is described in United States Patent No. 4,873,191. In yet another such method, embryonic cells are infected with a retrovirus containing the transgene whereby the germ cells of the embryo have the transgene chromosomally integrated therein. When the animals to be made transgenic are avian, because avian fertilized ova generally go
10 through cell division for the first twenty hours in the oviduct, microinjection into the pronucleus of the fertilized egg is problematic due to the inaccessibility of the pronucleus. Therefore, of the methods to make transgenic animals described generally above, retrovirus infection is preferred for avian species, for example as described in U.S. 5,162,215. If microinjection is to be used with avian species, however, a recently
15 published procedure by Love *et al.*, (Biotechnology, 12, Jan 1994) can be utilized whereby the embryo is obtained from a sacrificed hen approximately two and one-half hours after the laying of the previous laid egg, the transgene is microinjected into the cytoplasm of the germinal disc and the embryo is cultured in a host shell until maturity. When the animals to be made transgenic are bovine or porcine, microinjection can be
20 hampered by the opacity of the ova thereby making the nuclei difficult to identify by traditional differential interference-contrast microscopy. To overcome this problem, the ova can first be centrifuged to segregate the pronuclei for better visualization.

The "non-human animals" of the invention bovine, porcine, ovine and avian animals (e.g., cow, pig, sheep, chicken, turkey). The "transgenic non-human animals" of the
25 invention are produced by introducing "transgenes" into the germline of the non-human animal. Embryonal target cells at various developmental stages can be used to introduce transgenes. Different methods are used depending on the stage of development of the embryonal target cell. The zygote is the best target for micro-injection. The use of zygotes as a target for gene transfer has a major advantage in that in most cases the
30 injected DNA will be incorporated into the host gene before the first cleavage (Brinster

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et al., *Proc. Natl. Acad. Sci. USA* 82:4438-4442, 1985). As a consequence, all cells of the transgenic non-human animal will carry the incorporated transgene. This will in general also be reflected in the efficient transmission of the transgene to offspring of the founder since 50% of the germ cells will harbor the transgene.

- 5 The term "transgenic" is used to describe an animal which includes exogenous genetic material within all of its cells. A "transgenic" animal can be produced by cross-breeding two chimeric animals which include exogenous genetic material within cells used in reproduction. Twenty-five percent of the resulting offspring will be transgenic *i.e.*, animals which include the exogenous genetic material within all of their cells in both
10 alleles. 50% of the resulting animals will include the exogenous genetic material within one allele and 25% will include no exogenous genetic material.

In the microinjection method useful in the practice of the subject invention, the transgene is digested and purified free from any vector DNA *e.g.* by gel electrophoresis. It is preferred that the transgene include an operatively associated promoter which interacts
15 with cellular proteins involved in transcription, ultimately resulting in constitutive expression. Promoters useful in this regard include those from cytomegalovirus (CMV), Moloney leukemia virus (MLV), and herpes virus, as well as those from the genes encoding metallothionin, skeletal actin, P-enolpyruvate carboxylase (PEPCK), phosphoglycerate (PGK), DHFR, and thymidine kinase. Promoters for viral long
20 terminal repeats (LTRs) such as Rous Sarcoma Virus can also be employed. When the animals to be made transgenic are avian, preferred promoters include those for the chicken β -globin gene, chicken lysozyme gene, and avian leukosis virus. Constructs useful in plasmid transfection of embryonic stem cells will employ additional regulatory elements well known in the art such as enhancer elements to stimulate transcription,
25 splice acceptors, termination and polyadenylation signals, and ribosome binding sites to permit translation.

Retroviral infection can also be used to introduce transgene into a non-human animal, as described above. The developing non-human embryo can be cultured *in vitro* to the blastocyst stage. During this time, the blastomeres can be targets for retro viral infection (Jaenich, R., Proc. Natl. Acad. Sci USA 73:1260-1264, 1976). Efficient infection of the blastomeres is obtained by enzymatic treatment to remove the zona pellucida (Hogan, *et al.* (1986) in *Manipulating the Mouse Embryo*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.). The viral vector system used to introduce the transgene is typically a replication-defective retro virus carrying the transgene (Jahner, *et al.*, Proc. Natl. Acad. Sci. USA 82:6927-6931, 1985; Van der Putten, *et al.*, Proc. Natl. Acad. Sci USA 82:6148-6152, 1985). Transfection is easily and efficiently obtained by culturing the blastomeres on a monolayer of virus-producing cells (Van der Putten, *supra*; Stewart, *et al.*, EMBO J. 6:383-388, 1987). Alternatively, infection can be performed at a later stage. Virus or virus-producing cells can be injected into the blastocoele (D. Jahner *et al.*, Nature 298:623-628, 1982). Most of the founders will be mosaic for the transgene since incorporation occurs only in a subset of the cells which formed the transgenic nonhuman animal. Further, the founder may contain various retro viral insertions of the transgene at different positions in the genome which generally will segregate in the offspring. In addition, it is also possible to introduce transgenes into the germ line, albeit with low efficiency, by intrauterine retroviral infection of the midgestation embryo (D. Jahner *et al.*, *supra*).

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"Transformed" means a cell into which (or into an ancestor of which) has been introduced, by means of recombinant nucleic acid techniques, a heterologous nucleic acid molecule. "Heterologous" refers to a nucleic acid sequence that either originates from another species or is modified from either its original form or the form primarily
5 expressed in the cell.

"Transgene" means any piece of DNA which is inserted by artifice into a cell, and becomes part of the genome of the organism (*i.e.*, either stably integrated or as a stable extrachromosomal element) which develops from that cell. Such a transgene may include a gene which is partly or entirely heterologous (*i.e.*, foreign) to the transgenic
10 organism, or may represent a gene homologous to an endogenous gene of the organism. Included within this definition is a transgene created by the providing of an RNA sequence which is transcribed into DNA and then incorporated into the genome. The transgenes of the invention include DNA sequences which encode GDF-8, and include GDF-sense, antisense, dominant negative encoding polynucleotides, which may be
15 expressed in a transgenic non-human animal. The term "transgenic" as used herein additionally includes any organism whose genome has been altered by *in vitro* manipulation of the early embryo or fertilized egg or by any transgenic technology to induce a specific gene knockout. The term "gene knockout" as used herein, refers to the targeted disruption of a gene *in vivo* with complete loss of function that has been
20 achieved by any transgenic technology familiar to those in the art. In one embodiment, transgenic animals having gene knockouts are those in which the target gene has been rendered nonfunctional by an insertion targeted to the gene to be rendered non-functional by homologous recombination. As used herein, the term "transgenic" includes any transgenic technology familiar to those in the art which can produce an organism
25 carrying an introduced transgene or one in which an endogenous gene has been rendered non-functional or "knocked out." An example of a transgene used to "knockout" GDF-8 function in the present Examples is described in Example 8 and FIGURE 12a. Thus, in another embodiment, the invention provides a transgene wherein the entire mature C-terminal region of GDF-8 is deleted.

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The transgene to be used in the practice of the subject invention is a DNA sequence comprising a modified GDF-8 coding sequence. In a preferred embodiment, the GDF-8 gene is disrupted by homologous targeting in embryonic stem cells. For example, the entire mature C-terminal region of the GDF-8 gene may be deleted as described in the examples below. Optionally, the GDF-8 disruption or deletion may be accompanied by insertion of or replacement with other DNA sequences, such as a non-functional GDF-8 sequence. In other embodiments, the transgene comprises DNA antisense to the coding sequence for GDF-8. In another embodiment, the transgene comprises DNA encoding an antibody or receptor peptide sequence which is able to bind to GDF-8. The DNA and peptide sequences of GDF-8 are known in the art, the sequences, localization and activity disclosed in WO94/21681 and pending United States patent application 08/033,923, filed on March 19, 1993, incorporated by reference in its entirety. The disclosure of both of these applications are hereby incorporated herein by reference. Where appropriate, DNA sequences that encode proteins having GDF-8 activity but differ in nucleic acid sequence due to the degeneracy of the genetic code may also be used herein, as may truncated forms, allelic variants and interspecies homologues.

The invention also includes animals having heterozygous mutations in GDF-8 or partial inhibition of GDF-8 function or expression. A heterozygote would exhibit an intermediate increase in muscle and/or bone mass as compared to the homozygote as shown in Table 4 below. In other words, partial loss of function leads to a partial increase in muscle and bone mass. One of skill in the art would readily be able to determine if a particular mutation or if an antisense molecule was able to partially inhibit GDF-8. For example, *in vitro* testing may be desirable initially by comparison with wild-type or untreated GDF-8 (*e.g.*, comparison of northern blots to examine a decrease in expression).

After an embryo has been microinjected, colonized with transfected embryonic stem cells or infected with a retrovirus containing the transgene (except for practice of the subject invention in avian species which is addressed elsewhere herein) the embryo is implanted into the oviduct of a pseudopregnant female. The consequent progeny are tested for

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incorporation of the transgene by Southern blot analysis of blood samples using transgene specific probes. PCR is particularly useful in this regard. Positive progeny (G0) are crossbred to produce offspring (G1) which are analyzed for transgene expression by Northern blot analysis of tissue samples. To be able to distinguish
5 expression of like-species transgenes from expression of the animals endogenous GDF-8 gene(s), a marker gene fragment can be included in the construct in the 3' untranslated region of the transgene and the Northern probe designed to probe for the marker gene fragment. The serum levels of GDF-8 can also be measured in the transgenic animal to establish appropriate expression. Expression of the GDF-8 transgenes, thereby
10 decreasing the GDF-8 in the tissue and serum levels of the transgenic animals and consequently increasing the muscle tissue or bone tissue content results in the foodstuffs from these animals (*i.e.* eggs, beef, pork, poultry meat, milk, *etc.*) having markedly increased muscle and/or bone content, such as ribs, and preferably without increased, and more preferably, reduced levels of fat and cholesterol. By practice of the subject
15 invention, a statistically significant increase in muscle content, preferably at least a 2% increase in muscle content (*e.g.*, in chickens), more preferably a 25% increase in muscle content as a percentage of body weight, more preferably greater than 40% increase in muscle content in these foodstuffs can be obtained. Similarly the subject invention may provide a significant increase in bone content, such as ribs, in these foodstuffs.

20 **Additional Methods of Use**

Thus, the present invention includes methods for increasing muscle and bone mass in domesticated animals, characterized by inactivation or deletion of the gene encoding growth and differentiation factor-8 (GDF-8). The domesticated animal is preferably selected from the group consisting of ovine, bovine, porcine, piscine and avian. The
25 animal may be treated with an isolated polynucleotide sequence encoding growth and differentiation factor-8 which polynucleotide sequence is also from a domesticated animal selected from the group consisting of ovine, bovine, porcine, piscine and avian. The present invention includes methods for increasing the muscle and/or bone mass in

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domesticated animals characterized by administering to a domesticated animal monoclonal antibodies directed to the GDF-8 polypeptide. The antibody may be an anti-GDF-8, and may be either a monoclonal antibody or a polyclonal antibody.

The invention includes methods comprising using an anti-GDF-8 monoclonal antibody, antisense, or dominant negative mutants as a therapeutic agent to inhibit the growth regulating actions of GDF-8 on muscle and bone cells. Muscle and bone cells are defined to include fetal or adult muscle cells, as well as progenitor cells which are capable of differentiation into muscle or bone. The monoclonal antibody may be a humanized (*e.g.*, either fully or a chimeric) monoclonal antibody, of any species origin, such as murine, ovine, bovine, porcine or avian. Methods of producing antibody molecules with various combinations of "humanized" antibodies are well known in the art and include combining murine variable regions with human constant regions (Cabily, *et al. Proc.Natl.Acad.Sci. USA*, 81:3273, 1984), or by grafting the murine-antibody complementary determining regions (CDRs) onto the human framework (Richmann, *et al., Nature* 332:323, 1988). Other general references which teach methods for creating humanized antibodies include Morrison, *et al., Science*, 229:1202, 1985; Jones, *et al., Nature*, 321:522, 1986; Monroe, *et al., Nature* 312:779, 1985; Oi, *et al., BioTechniques*, 4:214, 1986; European Patent Application No. 302,620; and U.S. Patent No. 5,024,834. Therefore, by humanizing the monoclonal antibodies of the invention for *in vivo* use, an immune response to the antibodies would be greatly reduced.

The monoclonal antibody, GDF-8 polypeptide, or GDF-8 polynucleotide (all "GDF-8 agents") may have the effect of increasing the development of skeletal muscles and bones, such as ribs. In preferred embodiments of the claimed methods, the GDF-8 monoclonal antibody, polypeptide, or polynucleotide is administered to a patient suffering from a disorder selected from the group consisting of muscle wasting disease, neuromuscular disorder, muscle atrophy, bone degenerative diseases, osteoporosis, renal disease or aging. The GDF-8 agent may also be administered to a patient suffering from a disorder selected from the group consisting of muscular dystrophy, spinal cord injury, traumatic injury, congestive obstructive pulmonary disease (COPD), AIDS or cachexia.

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In a preferred embodiment, the GDF-8 agent is administered to a patient suffering from any of these diseases by intravenous, intramuscular or subcutaneous injection; preferably, a monoclonal antibody is administered within a dose range between about 0.1 mg/kg to about 100 mg/kg; more preferably between about 1 ug/kg to 75 mg/kg; most preferably
5 from about 10 mg/kg to 50 mg/kg. The antibody may be administered, for example, by bolus injection or by slow infusion. Slow infusion over a period of 30 minutes to 2 hours is preferred. The GDF-8 agent may be formulated in a formulation suitable for administration to a patient. Such formulations are known in the art.

The dosage regimen will be determined by the attending physician considering various
10 factors which modify the action of the GDF-8 protein, *e.g.* amount of tissue desired to be formed, the site of tissue damage, the condition of the damaged tissue, the size of a wound, type of damaged tissue, the patient's age, sex, and diet, the severity of any infection, time of administration and other clinical factors. The dosage may vary with the type of matrix used in the reconstitution and the types of agent, such as anti-GDF-8
15 antibodies, to be used in the composition. Generally, systemic or injectable administration, such as intravenous (IV), intramuscular (IM) or subcutaneous (Sub-Q) injection. Administration will generally be initiated at a dose which is minimally effective, and the dose will be increased over a preselected time course until a positive effect is observed. Subsequently, incremental increases in dosage will be made limiting such incremental
20 increases to such levels that produce a corresponding increase in effect, while taking into account any adverse effects that may appear. The addition of other known growth factors, such as IGF I (insulin like growth factor I), human, bovine, or chicken growth hormone which may aid in increasing muscle and bone mass, to the final composition, may also affect the dosage. In the embodiment where an anti-GDF-8 antibody is
25 administered, the anti-GDF-8 antibody is generally administered within a dose range of about 0.1 ug/kg to about 100 mg/kg.; more preferably between about 10 mg/kg to 50 mg/kg.

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Progress can be monitored by periodic assessment of tissue growth and/or repair. The progress can be monitored, for example, x-rays, histomorphometric determinations and tetracycline labeling.

Screening for GDF-8 Modulating Compounds

- 5 In another embodiment, the invention provides a method for identifying a compound or molecule that modulates GDF-8 protein activity or gene expression. The method includes incubating components comprising the compound, GDF-8 polypeptide or with a recombinant cell expressing GDF-8 polypeptide, under conditions sufficient to allow the components to interact and determining the effect of the compound on GDF-8
10 activity or expression. The effect of the compound on GDF-8 activity can be measured by a number of assays, and may include measurements before and after incubating in the presence of the compound. Compounds that affect GDF-8 activity or gene expression include peptides, peptidomimetics, polypeptides, chemical compounds and biologic agents. Assays include Northern blot analysis of GDF-8 mRNA (for gene expression),
15 Western blot analysis (for protein level) and muscle fiber analysis (for protein activity).

- The above screening assays may be used for detecting the compounds or molecules that bind to the GDF-8 receptor or GDF-8 polypeptide, in isolating molecules that bind to the GDF-8 gene, for measuring the amount of GDF-8 in a sample, either polypeptide or RNA (mRNA), for identifying molecules that may act as agonists or antagonists, and the like.
20 For example, GDF-8 antagonists are useful for treatment of muscular and adipose tissue disorders (e.g., obesity).

- Incubating includes conditions which allow contact between the test compound and GDF-8 polypeptide or with a recombinant cell expressing GDF-8 polypeptide. Contacting includes in solution and in solid phase, or in a cell. The test compound may
25 optionally be a combinatorial library for screening a plurality of compounds. Compounds identified in the method of the invention can be further evaluated, detected, cloned, sequenced, and the like, either in solution or after binding to a solid support, by any method usually applied to the detection of a specific DNA sequence such as PCR,

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oligomer restriction (Saiki, *et al.*, *Bio/Technology*, 3:1008-1012, 1985), allele-specific oligonucleotide (ASO) probe analysis (Conner, *et al.*, *Proc. Natl. Acad. Sci. USA*, 80:278, 1983), oligonucleotide Landegren, *et al.*, *Science*, 241:1077, 1988), and the like. Molecular techniques for DNA analysis have been reviewed (Landegren, *et al.*, *Science*,
 5 242:229-237, 1988).

All references cited herein are hereby incorporated by reference in their entirety.

The following examples are intended to illustrate but not limit the invention. While they are typical of those that might be used, other procedures known to those skilled in the art may alternatively be used.

10

EXAMPLE 1

IDENTIFICATION AND ISOLATION OF A NOVEL

TGF- β FAMILY MEMBER

To identify a new member of the TGF- β superfamily, degenerate oligonucleotides were designed which corresponded to two conserved regions among the known family
 15 members: one region spanning the two tryptophan residues conserved in all family members except MIS and the other region spanning the invariant cysteine residues near the C-terminus. These primers were used for polymerase chain reactions on mouse genomic DNA followed by subcloning the PCR products using restriction sites placed at the 5' ends of the primers, picking individual *E. coli* colonies carrying these subcloned
 20 inserts, and using a combination of random sequencing and hybridization analysis to eliminate known members of the superfamily.

GDF-8 was identified from a mixture of PCR products obtained with the primers
 SJL141: 5'-CCGGAATTCGGITGG(G/C/A)A(G/A/T/C)(A/G)A(T/C)TGG(A/G)TI
 (A/G)TI(T/G)CICC-3' (SEQ ID NO:1)

25 SJL147:

5'-CCGGAATTC(G/A)CAI(G/C)C(G/A)CA(G/A)CT(GIA/T/C)
 TCIACI(G/A)(T/C)CAT-3' (SEQ ID NO:2)

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PCR using these primers was carried out with 2 µg mouse genomic DNA at 94°C for 1 min, 50°C for 2 min, and 72°C for 2 min for 40 cycles.

PCR products of approximately 280 bp were gel-purified, digested with Eco RI, gel-purified again, and subcloned in the Bluescript vector (Stratagene, San Diego, CA).

- 5 Bacterial colonies carrying individual subclones were picked into 96 well microtiter plates, and multiple replicas were prepared by plating the cells onto nitrocellulose. The replicate filters were hybridized to probes representing known members of the family, and DNA was prepared from nonhybridizing colonies for sequence analysis.

- The primer combination of SJL141 and SJL147, encoding the amino acid sequences
10 GW(H/Q/N/K/D/E)(D/N)W(V/I/M)(V/I/M)(A/S)P (SEQ ID NO:9) and
M(V/I/M/T/A)V(D/E)SC(G/A)C (SEQ ID NO:10), respectively, yielded four previously identified sequences (BMP-4, inhibin,βB, GDF-3 and GDF-5) and one novel sequence, which was designated GDF-8, among 110 subclones analyzed.

Human GDF-8 was isolated using the primers:

- 15 ACM13: 5'-CGCGGATCCAGAGTCAAGGTGACAGACACAC-3' (SEQ ID NO:3); and
ACM14: 5'-CGCGGATCCTCCTCATGAGCACCCACAGCGGTC-3' (SEQ ID NO:4)

- PCR using these primers was carried out with one µg human genomic DNA at 94°C for 1 min, 58°C for 2 min, and 72°C for 2 min for 30 cycles. The PCR product was digested with Bam HI, gel-purified, and subcloned in the Bluescript vector (Stratagene, San
20 Francisco, CA).

EXAMPLE 2

EXPRESSION PATTERN AND SEQUENCE OF GDF-8

- To determine the expression pattern of GDF-8, RNA samples prepared from a variety of adult tissues were screened by Northern analysis. RNA isolation and Northern analysis
25 were carried out as described previously (Lee, S.J., *Mol. Endocrinol.*, 4:1034, 1990)

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except that hybridization was carried out in 5X SSPE, 10% dextran sulfate, 50% formamide, 1% SDS, 200 µg/ml salmon DNA, and 0.1% each of bovine serum albumin, ficoll, and polyvinylpyrrolidone. Five micrograms of twice poly A-selected RNA prepared from each tissue (except for muscle, for which only 2 µg RNA was used) were
5 electrophoresed on formaldehyde gels, blotted, and probed with GDF-8. As shown in FIGURE 1, the GDF-8 probe detected a single mRNA species expressed at highest levels in muscle and at significantly lower levels in adipose tissue.

To obtain a larger segment of the GDF-8 gene, a mouse genomic library was screened with a probe derived from the GDF-8 PCR product. The partial sequence of a GDF-8
10 genomic clone is shown in FIGURE 2a. The sequence contains an open reading frame corresponding to the predicted C-terminal region of the GDF-8 precursor protein. The predicted GDF-8 sequence contains two potential proteolytic processing sites, which are boxed. Cleavage of the precursor at the second of these sites would generate a mature C terminal fragment 109 amino acids in length with a predicted molecular weight of
15 12,400. The partial sequence of human GDF-8 is shown in FIGURE 2b. Assuming no PCR-induced errors during the isolation of the human clone, the human and mouse amino acid sequences in this region are 100% identical.

The C-terminal region of GDF-8 following the putative proteolytic processing site shows significant homology to the known members of the TGF-β₃ superfamily (FIGURE 3).
20 FIGURE 3 shows the alignment of the C-terminal sequences of GDF-8 with the corresponding regions of human GDF-1 (Lee, *Proc. Natl. Acad. Sci. USA*, 88:4250-4254, 1991), human BMP-2 and 4 (Wozney, *et al.*, *Science*, 242:1528-1534, 1988), human Vgr-1 (Celeste, *et al.* *Proc. Natl. Acad. Sci. USA*, 87:9843-9847, 1990), human OP-1 (Ozkaynak, *et al.*, *EMBO J.*, 9:2085-2093, 1990), human BMP-5 (Celeste, *et al.*, *Proc.*
25 *Natl. Acad. Sci. USA*, 87:9843-9847, 1990), human BMP-3 (Wozney, *et al.*, *Science*, 242:1528-1534, 1988), human MiS (Cate, *et al.* *Cell*, 45:685-698, 1986), human inhibin alpha, βA, and βB (Mason, *et al.*, *Biochem. Biophys. Res. Commun.*, 135:957-964, 1986), human TGF-β1 (Derynck, *et al.*, *Nature*, 316:701 -705, 1985), human TGF-R2 (deMartin, *et al.*, *EMBO J.*, 6:3673-3677, 1987), and human TGF-β3 (ten Dijke, *et al.*, *Proc. Natl.*

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Acad. Sci. USA, 85:4715-4719, 1988). The conserved cysteine residues are boxed. Dashes denote gaps introduced in order to maximize the alignment.

GDF-8 contains most of the residues that are highly conserved in other family members, including the seven cysteine residues with their characteristic spacing. Like the TGF- β s
5 and inhibin β s, GDF-8 also contains two additional cysteine residues. In the case of TGF- β 2, these two additional cysteine residues are known to form an intramolecular disulfide bond (Daopin, *et al.*, *Science*, 257:369, 1992; Schlunegger and Grutter, *Nature*, 358:430, 1992).

FIGURE 4 shows the amino acid homologies among the different members of the TGF- β
10 superfamily. Numbers represent percent amino acid identities between each pair calculated from the first conserved cysteine to the C terminus. Boxes represent homologies among highly-related members within particular subgroups. In this region, GDF-8 is most homologous to Vgr-1 (45% sequence identity).

EXAMPLE 3

15 ISOLATION OF cDNA CLONES ENCODING MURINE AND HUMAN GDF-8

In order to isolate full-length cDNA clones encoding murine and human GDF-8, cDNA libraries were prepared in the lambda ZAP II vector (Stratagene) using RNA prepared from skeletal muscle. From 5 μ g of twice poly A-selected RNA prepared from murine and human muscle, cDNA libraries consisting of 4.4 million and 1.9 million recombinant
20 phage, respectively, were constructed according to the instructions provided by Stratagene. These libraries were screened without amplification. Library screening and characterization of cDNA inserts were carried out as described previously (Lee, *Mol. Endocrinol.*, 4:1034-1040).

From 2.4×10^6 recombinant phage screened from the murine muscle cDNA library,
25 greater than 280 positive phage were identified using a murine GDF-8 probe derived from a genomic clone, as described in Example 1. The entire nucleotide sequence of the

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longest cDNA insert analyzed is shown in FIGURE 5a and 5b and SEQ ID NO:11. The 2676 base pair sequence contains a single long open reading frame beginning with a methionine codon at nucleotide 104 and extending to a TGA stop codon at nucleotide 1232. Upstream of the putative initiating methionine codon is an in-frame stop codon at nucleotide 23. The predicted pre-pro-GDF-8 protein is 76 amino acids in length. The sequence contains a core of hydrophobic amino acids at the N-terminus suggestive of a signal peptide for secretion (FIGURE 6a), one potential N-glycosylation site at asparagine 72, a putative RXXR proteolytic cleavage site at amino acids 264-267, and a C-terminal region showing significant homology to the known members of the TGF- β superfamily. Cleavage of the precursor protein at the putative RXXR site would generate a mature C-terminal GDF-8 fragment 109 amino acids in length with a predicted molecular weight of approximately 12,400.

From 1.9×10^6 recombinant phage screened from the human muscle cDNA library, 4 positive phage were identified using a human GDF-8 probe derived by polymerase chain reaction on human genomic DNA. The entire nucleotide sequence of the longest cDNA insert is shown in FIGURE 5c and 5d and SEQ ID NO:13. The 2743 base pair sequence contains a single long open reading frame beginning with a methionine codon at nucleotide 59 and extending to a TGA stop codon at nucleotide 1184. The predicted pre-pro-GDF-8 protein is 375 amino acids in length. The sequence contains a core of hydrophobic amino acids at the N-terminus suggestive of a signal peptide for secretion (FIGURE 6b), one potential N-glycosylation site at asparagine 71, and a putative RX(R) proteolytic cleavage site at amino acids 263-266. FIGURE 7 shows a comparison of the predicted murine (top) and human (bottom) GDF-8 amino acid sequences. Numbers indicate amino acid position relative to the N-terminus. Identities between the two sequences are denoted by a vertical line. Murine and human GDF-8 are approximately 94% identical in the predicted pro-regions and 100% identical following the predicted RXXR cleavage sites.

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EXAMPLE 4
DIMERIZATION OF GDF-8

To determine whether the processing signals in the GDF-8 sequence are functional and whether GDF-8 forms dimers like other members of the TGF- β superfamily, the GDF-8 cDNA was stably expressed in CHO cells. The GDF-8 coding sequence was cloned into the pMSXND expression vector (Lee and Nathans, *J. Biol. Chem.*, 263:3521,(1988) and transfected into CHO cells. Following G418 selection, the cells were selected in 0.2 μ M methotrexate, and conditioned medium from resistant cells was concentrated and electrophoresed on SDS gels. Conditioned medium was prepared by Cell Trends, Inc. (Middletown, MD). For preparation of anti-GDF-8 serum, the C-terminal region of GDF-8 (amino acids 268 to 376) was expressed in bacteria using the RSET vector (Invitrogen, San Diego, CA), purified using a nickle chelate column, and injected into rabbits. All immunizations were carried out by Spring Valley Labs (Woodbine, MD). Western analysis using [¹²⁵I]iodoprotein A was carried out as described (Burnette, W.N., *Anal. Biochem.*, 112:195, 1981). Western analysis of conditioned medium prepared from these cells using an antiserum raised against a bacterially-expressed C-terminal fragment of GDF-8 detected two protein species with apparent molecular weights of approximately 52K and 15K under reducing conditions, consistent with unprocessed and processed forms of GDF-8, respectively. No bands were obtained either with preimmune serum or with conditioned medium from CHO cells transfected with an antisense construct. Under non-reducing conditions, the GDF-8 antiserum detected two predominant protein species with apparent molecular weights of approximately 101K and 25K, consistent with dimeric forms of unprocessed and processed GDF-8, respectively. Hence, like other TGF- β family members, GDF-8 appears to be secreted and proteolytically processed, and the C-terminal region appears to be capable of forming a disulfide-linked dimer.

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EXAMPLE 5**PREPARATION OF ANTIBODIES AGAINST GDF-8 AND
EXPRESSION OF GDF-8 IN MAMMALIAN CELLS**

- In order to prepare antibodies against GDF-8, GDF-8 antigen was expressed as a fusion protein in bacteria. A portion of murine GDF-8 cDNA spanning amino acids 268-376 (mature region) was inserted into the pRSET vector (Invitrogen) such that the GDF-8 coding sequence was placed in frame with the initiating methionine codon present in the vector; the resulting construct created an open reading frame encoding a fusion protein with a molecular weight of approximately 16,600. The fusion construct was transformed into BL21 (DE3) (pLysS) cells, and expression of the fusion protein was induced by treatment with isopropylthio- β -galactoside as described (Rosenberg, *et al.*, *Gene*, 56:125-135). The fusion protein was then purified by metal chelate chromatography according to the instructions provided by Invitrogen. A Coomassie blue-stained gel of unpurified and purified fusion proteins is shown in FIGURE 8.
- 15 The purified fusion protein was used to immunize both rabbits and chickens. Immunization of rabbits was carried out by Spring Valley Labs (Sykesville, MD), and immunization of chickens was carried out by HRP, Inc. (Denver, PA). Western analysis of sera both from immunized rabbits and from immunized chickens demonstrated the presence of antibodies directed against the fusion protein.
- 20 To express GDF-8 in mammalian cells, the murine GDF-8 cDNA sequence from nucleotides 48-1303 was cloned in both orientations downstream of the metallothionein I promoter in the pMSXND expression vector; this vector contains processing signals derived from SV40, a dihydrofolate reductase gene, and a gene conferring resistance to the antibiotic G418 (Lee and Nathans, *J. Biol. Chem.*, 263:3521-3527). The resulting
- 25 constructs were transfected into Chinese hamster ovary cells, and stable transfectants were selected in the presence of G418. Two milliliters of conditioned media prepared from the G418-resistant cells were dialyzed, lyophilized, electrophoresed under denaturing,

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reducing conditions, transferred to nitrocellulose, and incubated with anti-GDF-8 antibodies (described above) and [¹²⁵I]iodoprotein A.

As shown in FIGURE 9, the rabbit GDF-8 antibodies (at a 1:500 dilution) detected a protein of approximately the predicted molecular weight for the mature C-terminal
5 fragment of GDF-8 in the conditioned media of cells transfected with a construct in which GDF-8 had been cloned in the correct (sense) orientation with respect to the metallothionein promoter (lane 2); this band was not detected in a similar sample prepared from cells transfected with a control antisense construct (lane 1). Similar results were obtained using antibodies prepared in chickens. Hence, GDF-8 is secreted and
10 proteolytically processed by these transfected mammalian cells.

EXAMPLE 6

EXPRESSION PATTERN OF GDF-8

To determine the pattern of GDF-8, 5 µg of twice poly A-selected RNA prepared from a variety of murine tissue sources were subjected to Northern analysis. As shown in
15 FIGURE 10a (and as shown previously in Example 2), the GDF-8 probe detected a single mRNA species present almost exclusively in skeletal muscle among a large number of adult tissues surveyed. On longer exposures of the same blot, significantly lower but detectable levels of GDF-8 mRNA were seen in fat, brain, thymus, heart, and lung. Hence, these results confirm the high degree of specificity of GDF-8 expression in
20 skeletal muscle. GDF-8 mRNA was also detected in mouse embryos at both gestational ages (day 12.5 and day 18.5 post-coital) examined but not in placentas at various stages of development (FIGURE 10b).

To further analyze the expression pattern of GDF-8, *in situ* hybridization was performed on mouse embryos isolated at various stages of development.

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For all *in situ* hybridization experiments, probes corresponding to the C-terminal region of GDF-8 were excluded in order to avoid possible cross-reactivity with other members of the superfamily. Whole mount *in situ* hybridization analysis was carried out as described (Wilkinson, D.G., *In Situ Hybridization, A Practical Approach*, pp. 75-83, 5 IRL Press, Oxford, 1992) except that blocking and antibody incubation steps were carried out as in Knecht *et al.* (Knecht, *et al.*, *Development*, 121:1927, 1995). Alkaline phosphatase reactions were carried out for 3 hours for day 10.5 embryos and overnight for day 9.5 embryos. Hybridization was carried out using digoxigenin-labelled probes spanning nucleotides 8-811 and 1298-2676, which correspond to the pro-region and 3' 10 untranslated regions, respectively. *In situ* hybridization to sections was carried out as described (Wilkinson, *et al.*, *Cell*, 50:79, 1987) using ³⁵S-labelled probes ranging from approximately 100-650 bases in length and spanning nucleotides 8-793 and 1566-2595. Following hybridization and washing, slides were dipped in NTB-3 photographic emulsion, exposed for 16-19 days, developed and stained with either hematoxylin and 15 eosin or toluidine blue. RNA isolation, poly A selection, and Northern analysis were carried out as described previously (McPherron and Lee, *J. Biol. Chem.*, 268:3444, 1993).

At all stages examined, the expression of GDF-8 mRNA appeared to be restricted to developing skeletal muscle. At early stages, GDF-8 expression was restricted to 20 developing somites. By whole mount *in situ* hybridization analysis, GDF-8 mRNA could first be detected as early as day 9.5 post coitum in approximately one-third of the somites. At this stage of development, hybridization appeared to be restricted to the most mature (9 out of 21 in this example), rostral somites. By day 10.5 p.c., GDF-8 expression was clearly evident in almost every somite (28 out of 33 in this example 25 shown). Based on *in situ* hybridization analysis of sections prepared from day 10.5 p.c. embryos, the expression of GDF-8 in somites appeared to be localized to the myotome compartment. At later stages of development, GDF-8 expression was detected in a wide range of developing muscles.

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GDF-8 continues to be expressed in adult animals as well. By Northern analysis, GDF-8 mRNA expression was seen almost exclusively in skeletal muscle among the different adult tissues examined. A significantly lower though clearly detectable signal was also seen in adipose tissue. Based on Northern analysis of RNA prepared from a large
5 number of different adult skeletal muscles, GDF-8 expression appeared to be widespread although the expression levels varied among individual muscles.

EXAMPLE 7

CHROMOSOMAL LOCALIZATION OF GDF-8

In order to map the chromosomal location of GDF-8, DNA samples from human/rodent
10 somatic cell hybrids (Drwina, *et al.*, *Genomics*, 16:311-413, 1993; Dubois and Naylor, *Genomics*, 16:315-319, 1993) were analyzed by polymerase chain reaction followed by Southern blotting. Polymerase chain reaction was carried out using primer #83, 5'-C-GCGGATCCGTGGATCTAAATGAGAACAGTGAGC-3' (SEQ ID NO: 15) and primer #84, 5'-CGCGAATTCTCAGGTAATGATTGTTTCCGTTGTAGCG-3' (SEQ ID NO:16)
15 for 40 cycles at 94°C for 2 minutes, 60°C for 1 minute, and 72°C for 2 minutes. These primers correspond to nucleotides 119 to 143 (flanked by a Bam H1 recognition sequence), and nucleotides 394 to 418 (flanked by an Eco R1 recognition sequence), respectively, in the human GDF-8 cDNA sequence. PCR products were electrophoresed on agarose gels, blotted, and probed with oligonucleotide #100,
20 5'-ACACTAAATCTTCAAGAATA-3' (SEQ ID NO:17), which corresponds to a sequence internal to the region flanked by primer #83 and #84. Filters were hybridized in 6 X SSC, 1 X Denhardt's solution, 100µg/ml yeast transfer RNA, and 0.05% sodium pyrophosphate at 50°C.

As shown in FIGURE 11, the human-specific probe detected a band of the predicted size
25 (approximately 320 base pairs) in the positive control sample (total human genomic DNA) and in a single DNA sample from the human/rodent hybrid panel. This positive signal corresponds to human chromosome 2. The human chromosome contained in each of the hybrid cell lines is identified at the top of each of the first 24 lanes (1-22, X, and

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Y). In the lanes designated M, CHO, and H, the starting DNA template was total genomic DNA from mouse, hamster, and human sources, respectively. In the lane marked B1, no template DNA was used. Numbers at left indicate the mobilities of DNA standards. These data show that the human GDF-8 gene is located on chromosome 2.

5

EXAMPLE 8

GDF-8 TRANSGENIC KNOCKOUT MICE

The GDF-8, we disrupted the GDF-8 gene was disrupted by homologous targeting in embryonic stem cells. To ensure that the resulting mice would be null for GDF-8 function, the entire mature C-terminal region was deleted and replaced by a neo cassette
10 (Figure 12a). A murine 129 SV/J genomic library was prepared in lambda FIX II according to the instructions provided by Stratagene (La Jolla, CA). The structure of the GDF-8 gene was deduced from restriction mapping and partial sequencing of phage clones isolated from this library. Vectors for preparing the targeting construct were kindly provided by Philip Soriano and Kirk Thomas University. R1 ES cells were trans-
15 fected with the targeting construct, selected with gancyclovir (2 μ M) and G418 (250 μ g/ml), and analyzed by Southern analysis. Homologously targeted clones were injected into C57BL/6 blastocysts and transferred into pseudopregnant females. Germline transmission of the targeted allele was obtained in a total of 9 male chimeras from 5 independently-derived ES clones. Genomic Southern blots were hybridized at 42°C as
20 described above and washed in 0.2X SSC, 0.1% SDS at 42°C.

For whole leg analysis, legs of 14 week old mice were skinned, treated with 0.2 M EDTA in PBS at 4°C for 4 weeks followed by 0.5 M sucrose in PBS at 4°C. For fiber number and size analysis, samples were directly mounted and frozen in isopentane as described (Brumback and Leech, *Color Atlas of Muscle Histochemistry*, pp. 9-33, PSG Publishing
25 Company, Littleton, MA, 1984). Ten to 30 μ m sections were prepared using a cryostat and stained with hematoxylin and eosin. Muscle fiber numbers were determined from sections taken from the widest part of the tibialis cranialis muscle. Muscle fiber sizes were measured from photographs of sections of tibialis cranialis and gastrocnemius muscles. Fiber type analysis was carried out using the mysosin ATPase assay after

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pretreatment at pH 4.35 as described (Cumming, *et al.*, *Color Atlas of Muscle Pathology*, pp. 184-185, 1994) and by immunohistochemistry using an antibody directed against type I myosin (MY32, Sigma) and the Vectastain method (Vector Labs); in the immunohistochemical experiments, no staining was seen when the primary antibodies
5 were left out. Carcasses were prepared from shaved mice by removing the all of the internal organs and associated fat and connective tissue. Fat content of carcasses from 4 month old males was determined as described (Leshner, *et al.*, *Physiol. Behavior*, 9:281, 1972).

For protein and DNA analysis, tissue was homogenized in 150 mM NaCl, 100 mM
10 EDTA. Protein concentrations were determined using the Biorad protein assay. DNA was isolated by adding SDS to 1%, treating with 1 mg/ml proteinase K overnight at 55°C, extracting 3 times with phenol and twice with chloroform, and precipitating with ammonium acetate and EtOH. DNA was digested with 2 mg/ml RNase for 1 hour at 37°C, and following proteinase K digestion and phenol and chloroform extractions, the
15 DNA was precipitated twice with ammonium acetate and EtOH.

Homologous targeting of the GDF-8 gene was seen in 13/131 gancyclovir/G418 doubly-resistant ES cell clones. Following injection of these targeted clones into blastocysts, we obtained chimeras from 5 independently-derived ES clones that produced heterozygous pups when crossed to C57BL/6 females (Figure 12b). Genotypic analysis
20 of 678 offspring derived from crosses of F1 heterozygotes showed 170 +/+ (25%), 380 +/- (56%), and 128 -/- (19%). Although the ratio of genotypes was close to the expected ratio of 1:2:1, the smaller than expected number of homozygous mutants appeared to be statistically significant ($p < 0.001$).

Homozygous mutants were viable and fertile when crossed to C57BL/6 mice and to each
25 other. Homozygous mutant animals, however, were approximately 30% larger than their heterozygous and wild type littermates (Table 1). The difference between mutant and wild type body weights appeared to be relatively constant irrespective of age and sex in adult animals. Adult mutants also displayed an abnormal body shape, with pronounced

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shoulders and hips. When the skin was removed from animals that had been sacrificed, it was apparent that the muscles of the mutants were much larger than those of wild type animals. The increase in skeletal muscle mass appeared to be widespread throughout the body. Individual muscles isolated from homozygous mutant animals weighed
5 approximately 2-3 times more than those isolated from wild type littermates (Table 2). Although the magnitude of the weight increase appeared to roughly correlate with the level of GDF-8 expression in the muscles examined. To determine whether the increased muscle mass could account for the entire difference in total body weights between wild type and mutant animals or whether many tissues were generally larger in the mutants,
10 we compared the total body weights to carcass weights. As shown in Table 3, the difference in carcass weights between wild type and mutant animals was comparable to the difference in total body weights. Moreover, because the fat content of mutant and wild type animals was similar, these data are consistent with all of the total body weight difference resulting from an increase in skeletal muscle mass, although we have not
15 formally ruled out the possibility that differences in bone mass might also contribute to the differences in total body mass.

To determine whether the increase in skeletal muscle mass resulted from hyperplasia or from hypertrophy, histologic analysis of several different muscle groups was performed. The mutant muscle appeared grossly normal. No excess connective tissue or fat was seen
20 nor were there any obvious signs of degeneration, such as widely varying fiber sizes (see below) or centrally-placed nuclei. Quantitation of the number of muscle fibers showed that at the widest portion of the tibialis cranialis muscle, the total cell number was 86% higher in mutant animals compared to wild type littermates [mutant = 5470 +/- 121 (n = 3), wild type = 2936 +/- 288 (n = 3); $p < 0.01$]. Consistent with this result was the
25 finding that the amount of DNA extracted from mutant muscle was roughly 50% higher than from wild type muscle [mutant = 350 μ g (n = 4), wild type = 233 μ g (n = 3) from pooled gastrocnemius, plantaris, triceps brachii, tibialis cranialis, and pectoralis muscles; $p = 0.05$]. Hence, a large part of the increase in skeletal muscle mass resulted from muscle cell hyperplasia. However, muscle fiber hypertrophy also appeared to contribute
30 to the overall increase in muscle mass. As shown in Figure 13, the mean fiber diameter

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of the tibialis cranialis muscle and gastrocnemius muscle was 7% and 22% larger, respectively, in mutant animals compared to wild type littermates, suggesting that the cross-sectional area of the fibers was increased by approximately 14% and 49%, respectively. Notably, although the mean fiber diameter was larger in the mutants, the standard deviation in fiber sizes was similar between mutant and wild type muscle, consistent with the absence of muscle degeneration in mutant animals. The increase in fiber size was also consistent with the finding that the protein to DNA ratio (w/w) was slightly increased in mutant compared to wild type muscle [mutant = 871 +/- 111 (n = 4), wild type = 624 +/- 85 (n = 3); $p < 0.05$].

10 Table 4 shows a comparison between muscle weight (in grams) from wild-type (+/+), heterozygous (+/-) and a homozygous knock-out mice (-/-). The muscle mass is increased in heterozygous as compared to wild-type animals.

Finally, fiber type analysis of various muscles was carried out to determine whether the number of both type I (slow) and type II (fast) fibers was increased in the mutant animals. In most of the muscles examined, including the tibialis cranialis muscle, the vast majority of muscle fibers were type II in both mutant and wild type animals. Hence, based on the cell counts discussed above, the absolute number of type II fibers were increased in the tibialis cranialis muscle. In the soleus muscle, where the number of type I fibers was sufficiently high that we could attempt to quantitate the ratio of fiber types could be quantiated, the percent of type I fibers was decreased by approximately 33% in mutant compared to wild type muscle [wild type = 39.2 +/- 8.1 (n = 3), mutant = 26.4 +/- 9.3 (n = 4)]; however, the variability in this ratio for both wild type and mutant animals was too high to support any firm conclusions regarding the relative number of fiber types.

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EXAMPLE 9**ISOLATION OF RAT AND CHICKEN GDF-8**

- In order to isolate rat and chicken GDF-8 cDNA clones, skeletal muscle cDNA libraries prepared from these species were obtained from Stratagene and screened with a murine
- 5 GDF-8 probe. Library screening was carried out as described previously (Lee, Mol. Endocrinol., 4:1034-1040) except that final washes were carried out in 2 X SSC at 65°C. Partial sequence analysis of hybridizing clones revealed the presence of open reading frames highly related to murine and human GDF-8. Partial sequences of rat and chicken
- 10 rat and chicken GDF-8 amino acid sequences with those of murine and human GDF-8 are shown in Figure 3b. Full length rat and chicken GDF-8 is shown in Figures 14d and 14c, respectively and sequence alignment between murine, rat, human, baboon, porcine, ovine, bovine, chicken, and turkey sequences is shown in Figures 15a and 15b. All
- 15 sequences contain an RSRR sequence that is likely to represent the proteolytic processing site. Following this RSRR sequence, the sequences contain a C-terminal region that is 100% conserved among all four species. The absolute conservation of the C-terminal region between species as evolutionarily far apart as humans and chickens, and baboons and turkeys, suggests that this region will be highly conserved in many other species as well.
- 20 Similar methodology was used to obtain the nucleotide and amino acid sequences for baboon (SEQ ID NO:18 and 19, respectively; Figure 14a); bovine (SEQ ID NO:20 and 21, respectively; Figure 14b); turkey (SEQ ID NO:26 and 27, respectively; Figure 14e); porcine (SEQ ID NO:28 and 29, respectively; Figure 14f); and ovine (SEQ ID NO:30 and 31, respectively; Figure 14g).

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EXAMPLE 10**GDF-11 HOMOLOGY IN MAMMALIAN SPECIES**

The overall homology between GDF-11 and GDF-8 based upon their respective amino acid sequence is approximately 92% (see for example, PCT/US95/08543, which is incorporated herein by reference). Thus, it is expected that animals expressing GDF-8 and GDF-11 will display similar phenotypes. Similarly, animals having a disruption in a GDF-8 or GDF-11 gene will display similar phenotypes. The relationship of GDF-8 to GDF-11 will be further understood in light of the following examples, in which GDF-11 knockout mice were created.

- 10 Like most other TGF- β family member, GDF-11 also appears to be highly conserved across species. By genomic Southern analysis, homologous sequences were detected in all mammalian species examined as well as in chickens and frogs (Figure 16). In most species, the GDF-11 probe also detected a second, more faintly hybridizing fragment corresponding to the myostatin gene (McPherron et al., 1997).

EXAMPLE 11**GDF-11 KNOCKOUT MICE**

- 15 To determine the biological function of GDF-11, we disrupted the GDF-11 gene by homologous targeting in embryonic stem cells. A murine 129 SV/J genomic library was prepared in lambda FIXII according to the instructions provided by Stratagene (La Jolla, CA). The structure of the GDF-11 gene was deduced from restriction mapping and partial sequencing of phage clones isolated from the library. Vectors for preparing the targeting construct were kindly provided by Philip Soriano and Kirk Thomas. To ensure that the resulting mice would be null for GDF-11 function, the entire mature C-terminal region was deleted and replaced by a neo cassette (Figure 17a,b). R1 ES cells were
- 20 transfected with the targeting construct, selected with gancyclovir (2 μ M) and G418 (250 μ g/ml), and analyzed by Southern analysis. Homologous targeting of the GDF-11 gene was seen in 8/155 gancyclovir/G418 doubly resistant ES cell clones. Following injection of several targeted clones into C57BL/6J blastocysts, we obtained chimeras from one ES clone that produced heterozygous pups when crossed to both C57BL/6J and 129/SvJ

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females. Crosses of C57BL/6J/129/SvJ hybrid F1 heterozygotes produced 49 wild-type (34%), 94 heterozygous (66%) and no homozygous mutant adult offspring. Similarly, there were no adult homozygous null animals seen in the 129/SvJ background (32 wild-type (36%) and 56 heterozygous mutant (64%) animals).

- 5 To determine the age at which homozygous mutants were dying, we genotyped litters of embryos isolated at various gestational ages from heterozygous females that had been mated to heterozygous males. At all embryonic stages examined, homozygous mutant embryos were present at approximately the predicted frequency of 25%. Among hybrid newborn mice, the different genotypes were also represented at the expected Mendelian
10 ratio of 1:2:1 (34 +/+ (28%), 61 +/- (50%), and 28 -/- (23%)). Homozygous mutant mice were born alive and were able to breath and nurse. All homozygous mutants died, however, within the first 24 hours after birth. The precise cause of death was unknown, but the lethality may have been related to the fact that the kidneys in homozygous mutants were either severely hypoplastic or completely absent. A summary of the
15 kidney abnormalities in these mice is shown in Figure 18.

EXAMPLE 12

ANATOMICAL DIFFERENCES IN GDF-11 KNOCKOUT MICE

- Homozygous mutant animals were easily recognizable by their severely shortened or absent tails (Figure 19a). To further characterize the tail defects in these homozygous
20 mutant animals, we examined their skeletons to determine the degree of disruption of the caudal vertebrae. A comparison of wild-type and mutant skeleton preparations of late stage embryos and newborn mice, however, revealed differences not only in the caudal region of the animals but in many other regions as well. In nearly every case where differences were noted, the abnormalities appeared to represent homeotic transformations
25 of vertebral segments in which particular segments appeared to have a morphology typical of more anterior segments. These transformations, which are summarized in Figure 20, were evident throughout the axial skeleton extending from the cervical region to the caudal region. Except for the defects seen in the axial skeleton, the rest of the skeleton, such as the cranium and limb bones, appeared normal.

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Anterior transformations of the vertebrae in mutant newborn animals were most readily apparent in the thoracic region, where there was a dramatic increase in the number of thoracic (T) segments. All wild-type mice examined showed the typical pattern of 13 thoracic vertebrae each with its associated pair of ribs (Figure 19(b,e)). In contrast, 5 homozygous mutant mice showed a striking increase in the number of thoracic vertebrae. All homozygous mutants examined had 4 to 5 extra pairs of ribs for a total of 17 to 18 (Figure 19(d,g)) although in over 1/3 of these animals, the 18th rib appeared to be rudimentary. Hence, segments that would normally correspond to lumbar (L) segments L1 to L4 or L5 appeared to have been transformed into thoracic segments in mutant 10 animals.

Moreover, transformations within the thoracic region in which one thoracic vertebra had a morphology characteristic of another thoracic vertebra were also evident. For example, in wild-type mice, the first 7 pairs of ribs attach to the sternum, and the remaining 6 are unattached or free (Figure 19(e,h)). In homozygous mutants, there was an increase in the 15 number of both attached and free pairs of ribs to 10-11 and 7-8, respectively (Figure 19(g,j)). Therefore, thoracic segments T8, T9, T10, and in some cases even T11, which all have free ribs in wild-type animals, were transformed in mutant animals to have a characteristic typical of more anterior thoracic segments, namely, the presence of ribs attached to the sternum. Consistent with this finding, the transitional spinous process and 20 transitional articular processes which are normally found on T10 in wild-type animals were instead found on T13 in homozygous mutants (data not shown). Additional transformations within the thoracic region were also noted in certain mutant animals. For example, in wild-type mice, the ribs derived from T1 normally touch the top of the sternum. However, in 2/23 hybrid and 2/3 129/SvJ homozygous mutant mice examined, 25 T2 appeared to have been transformed to have a morphology resembling that of T1; that is, in these animals, the ribs derived from T2 extended to touch the top of the sternum. In these cases, the ribs derived from T1 appeared to fuse to the second pair of ribs. Finally, in 82% of homozygous mutants, the long spinous process normally present on T2 was shifted to the position of T3. In certain other homozygous mutants, asymmetric 30 fusion of a pair of vertebrosteral ribs was seen at other thoracic levels.

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- The anterior transformations were not restricted to the thoracic region. The anterior most transformation that we observed was at the level of the 6th cervical vertebra (C6). In wild-type mice, C6 is readily identifiable by the presence of two anterior tuberculi on the ventral side. In several homozygous mutant mice, although one of these two anterior tuberculi was present on C6, the other was present at the position of C7 instead. Hence, in these mice, C7 appeared to have been partially transformed to have a morphology resembling that of C6. One other homozygous mutant had 2 anterior tuberculi on C7 but retained one on C6 for a complete C7 to C6 transformation but a partial C6 to C5 transformation.
- 10 Transformations of the axial skeleton also extended into the lumbar region. Whereas wild-type animals normally have only 6 lumbar vertebrae, homozygous mutants had 8-9. At least 6 of the lumbar vertebrae in the mutants must have derived from segments that would normally have given rise to sacral and caudal vertebrae as the data described above suggest that 4 to 5 lumbar segments were transformed into thoracic segments.
- 15 Hence, homozygous mutant mice had a total of 33-34 presacral vertebrae compared to 26 presacral vertebrae normally present in wild-type mice. The most common presacral vertebral patterns were C7/T18/L8 and C7/T18/L9 for mutant mice compared to C7/T13/L6 for wild-type mice. The presence of additional presacral vertebrae in mutant animals was obvious even without detailed examination of the skeletons as the position
- 20 of the hindlimbs relative to the forelimbs was displaced posteriorly by 7-8 segments.

- Although the sacral and caudal vertebrae were also affected in homozygous mutant mice, the exact nature of each transformation was not as readily identifiable. In wild-type mice, sacral segments S1 and S2 typically have broad transverse processes compared to S3 and S4. In the mutants, there did not appear to be an identifiable S1 or S2 vertebra.
- 25 Instead, mutant animals had several vertebrae that appeared to have morphology similar to S3. In addition, the transverse processes of all 4 sacral vertebrae are normally fused to each other although in newborns often only fusions of the first 3 vertebrae are seen. In homozygous mutants, however, the transverse processes of the sacral vertebrae were usually unfused. In the caudalmost region, all mutant animals also had severely

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malformed vertebrae with extensive fusions of cartilage. Although the severity of the fusions made it difficult to count the total number of vertebrae in the caudal region, we were able to count up to 15 transverse processes in several animals. We were unable to determine whether these represented sacral or caudal vertebrae in the mutants because
5 we could not establish morphologic criteria for distinguishing S4 from caudal vertebrae even in wild-type newborn animals. Regardless of their identities, the total number of vertebrae in this region was significantly reduced from the normal number of approximately 30. Hence, although the mutants had significantly more thoracic and lumbar vertebrae than wild-type mice, the total number of segments was reduced in the mutants
10 due to the truncation of the tails.

Heterozygous mice also showed abnormalities in the axial skeleton although the phenotype was much milder than in homozygous mice. The most obvious abnormality in heterozygous mice was the presence of an additional thoracic segment with an associated pair of ribs (Figure 19(c,f)). This transformation was present in every
15 heterozygous animal examined, and in every case, the additional pair of ribs was attached to the sternum (Figure 19(i)). Hence, T8, whose associated rib normally does not touch the sternum, appeared to have been transformed to a morphology characteristic of a more anterior thoracic vertebra, and L1 appeared to have been transformed to a morphology characteristic of a posterior thoracic vertebra. Other abnormalities indicative of anterior
20 transformations were also seen to varying degrees in heterozygous mice. These included a shift of the long spinous process characteristic of T2 by one segment to T3, a shift of the articular and spinous processes from T10 to T11, a shift of the anterior tuberculus on C6 to C7, and transformation of T2 to T1 where the rib associated with T2 touched the top of the sternum.

25 In order to understand the basis for the abnormalities in axial patterning seen in GDF-11 mutant mice, we examined mutant embryos isolated at various stages of development and compared them to wild-type embryos. By gross morphological examination, homozygous mutant embryos isolated up to day 9.5 of gestation were not readily distinguishable from corresponding wild-type embryos. In particular, the number of somites present at

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any given developmental age was identical between mutant and wild-type embryos, suggesting that the rate of somite formation was unaltered in the mutants. By day 10.5-11.5 p.c., mutant embryos could be easily distinguished from wild-type embryos by the posterior displacement of the hindlimb by 7-8 somites. The abnormalities in tail
5 development were also readily apparent at this stage. Taken together, these data suggest that the abnormalities observed in the mutant skeletons represented true transformations of segment identities rather than the insertion of additional segments, for example, by an enhanced rate of somitogenesis.

Alterations in expression of homeobox containing genes are known to cause transforma-
10 tions in Drosophila and in vertebrates. To see if the expression patterns of Hox genes (the vertebrate homeobox containing genes) were altered in GDF-11 null mutants we determined the expression pattern of 3 representative Hox genes, Hoxc-6, Hoxc-8 and Hoxc-11, in day 12.5 p.c. wild-type, heterozygous and homozygous mutant embryos by whole mount in situ hybridization. The expression pattern of Hoxc-6 in wild-type
15 embryos spanned prevertebrae 8-15 which correspond to thoracic segments T1-T8. In homozygous mutants, however, the Hoxc-6 expression pattern was shifted posteriorly and expanded to prevertebrae 9-18 (T2-T11). A similar shift was seen with the Hoxc-8 probe. In wild-type embryos, Hoxc-8 was expressed in prevertebrae 13-18 (T6-T11) but, in homozygous mutant embryos, Hoxc-8 was expressed in prevertebrae 14-22 (T7-T15).
20 Finally, Hoxc-11 expression was also shifted posteriorly in that the anterior boundary of expression changed from prevertebrae 28 in wild-type embryos to prevertebrae 36 in mutant embryos. (Note that because the position of the hindlimb is also shifted posteriorly in mutant embryos, the Hoxc-11 expression patterns in wild-type and mutant appeared similar relative to the hindlimbs). These data provide further evidence that the
25 skeletal abnormalities seen in mutant animals represent homeotic transformations.

The phenotype of GDF-11 mice suggested that GDF-11 acts early during embryogenesis as a global regulator of axial patterning. To begin to examine the mechanism by which GDF-11 exerts its effects, we determined the expression pattern of GDF-11 in early mouse embryos by whole mount in situ hybridization. At these stages the primary sites

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of GDF-11 expression correlated precisely with the known sites at which mesodermal cells are generated. Expression of GDF-11 was first detected at day 8.25-8.5 p.c. (8-10 somites) in the primitive streak region, which is the site at which ingressing cells form the mesoderm of the developing embryo. Expression was maintained in the primitive streak at day 8.75, but by day 9.5 p.c., when the tail bud replaces the primitive streak as the source of new mesodermal cells, expression of GDF-11 shifted to the tail bud. Hence at these early stages, GDF-11 appears to be synthesized in the region of the developing embryo where new mesodermal cells arise and presumably acquire their positional identity.

- 10 The phenotype of GDF-11 knockout mice in several respects resembles the phenotype of mice carrying a deletion of a receptor for some members of the TGF- β superfamily, the activin type IIB receptor (ActRIIB). As in the case of GDF-11 knockout mice, the ActRIIB knockout mice have extra pairs of ribs and a spectrum of kidney defects ranging from hypoplastic kidneys to complete absence of kidneys. The similarity in the
- 15 phenotypes of these mice raises the possibility that ActRIIB may be a receptor for GDF-11. However, ActRIIB cannot be the sole receptor for GDF-11 because the phenotype of GDF-11 knockout mice is more severe than the phenotype of ActRIIB mice. For example, whereas the GDF-11 knockout animals have 4-5 extra pairs of ribs and show homeotic transformations throughout the axial skeleton, the ActRIIB knockout
- 20 animals have only 3 extra pairs of ribs and do not show transformations at other axial levels. In addition, the data indicate that the kidney defects in the GDF-11 knockout mice are also more severe than those in ActRIIB knockout mice. The ActRIIB knockout mice show defects in left/right axis formation, such as lung isomerism and a range of heart defects that we have not yet observed in GDF-11 knockout mice. ActRIIB can bind
- 25 the activins and certain BMPs, although none of the knockout mice generated for these ligands show defects in left/right axis formation.

If GDF-11 does act directly on mesodermal cells to establish positional identity, the data presented here would be consistent with either short range or morphogen models for GDF-11 action. That is, GDF-11 may act on mesodermal precursors to establish patterns

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of Hox gene expression as these cells are being generated at the site of GDF-11 expression, or alternatively, GDF-11 produced at the posterior end of the embryo may diffuse to form a morphogen gradient. Whatever the mechanism of action of GDF-11 may be, the fact that gross anterior/posterior patterning still does occur in GDF-11
5 knockout animals suggests that GDF-11 may not be the sole regulator of anterior/posterior specification. Nevertheless, it is clear that GDF-11 plays an important role as a global regulator of axial patterning and that further study of this molecule will lead to important new insights into how positional identity along the anterior/posterior axis is established in the vertebrate embryo.

- 10 Similar phenotypes are expected in GDF-8 knockout animals. For example, GDF-8 knockout animals are expected to have increased number of ribs, kidney defects and anatomical differences when compared to wild-type.

Although the invention has been described with reference to the presently preferred embodiment, it should be understood that various modifications can be made without
15 departing from the spirit of the invention. Accordingly, the invention is limited only by the following claims.

CLAIMS

1. A method of producing animal food products having an increased number of ribs comprising:
 - a) introducing a transgene disrupting or interfering with expression of growth differentiation factor-8 (GDF-8) into an embryo into germ cells of a pronuclear embryo of the animal;
 - b) implanting the embryo into the oviduct of a pseudopregnant female thereby allowing the embryo to mature to full term progeny;
 - c) testing the progeny for presence of the transgene to identify transgene-positive progeny;
 - d) cross-breeding transgene-positive progeny to obtain further transgene-positive progeny; and
 - e) processing the progeny to obtain foodstuff.
2. The method of claim 1, wherein the transgene comprises GDF-8 antisense polynucleotides.
3. The method of claim 1, wherein the transgene comprises a gene encoding a dominant negative GDF-8 polypeptide.
4. A method of producing avian, porcine or bovine food products having an increased number of ribs comprising:
 - a) introducing a transgene disrupting or interfering with expression of growth differentiation factor-8 (GDF-8) into an embryo of an avian, porcine or bovine animal;
 - b) culturing the embryo under conditions whereby progeny are hatched;
 - c) testing the progeny for presence of the transgene to identify transgene-positive progeny;
 - d) cross-breeding transgene-positive progeny; and
 - e) processing the progeny to obtain foodstuff.

5. The method of claim 4, wherein the transgene comprises GDF-8 antisense polynucleotides.
6. The method of claim 4, wherein the transgene comprises a gene encoding a dominant negative GDF-8 polypeptide.
7. The transgenic animal of claim 4, wherein the transgene comprises a polynucleotide encoding a truncated GDF-8 polypeptide.
8. A method of treating a chronic or acute renal disease in a subject having such a disease, comprising:
administering to the subject, a reagent which affects GDF-8 activity or expression.
9. The method of Claim 8, wherein the reagent is an agonist of GDF-8.
10. The method of claim 8, wherein the reagent is an antagonist of GDF-8.
11. The method of claim 10, wherein the antagonist is an antibody to GDF-8.
12. The method of claim 10, wherein the antagonist is an antisense polynucleotide to GDF-8.

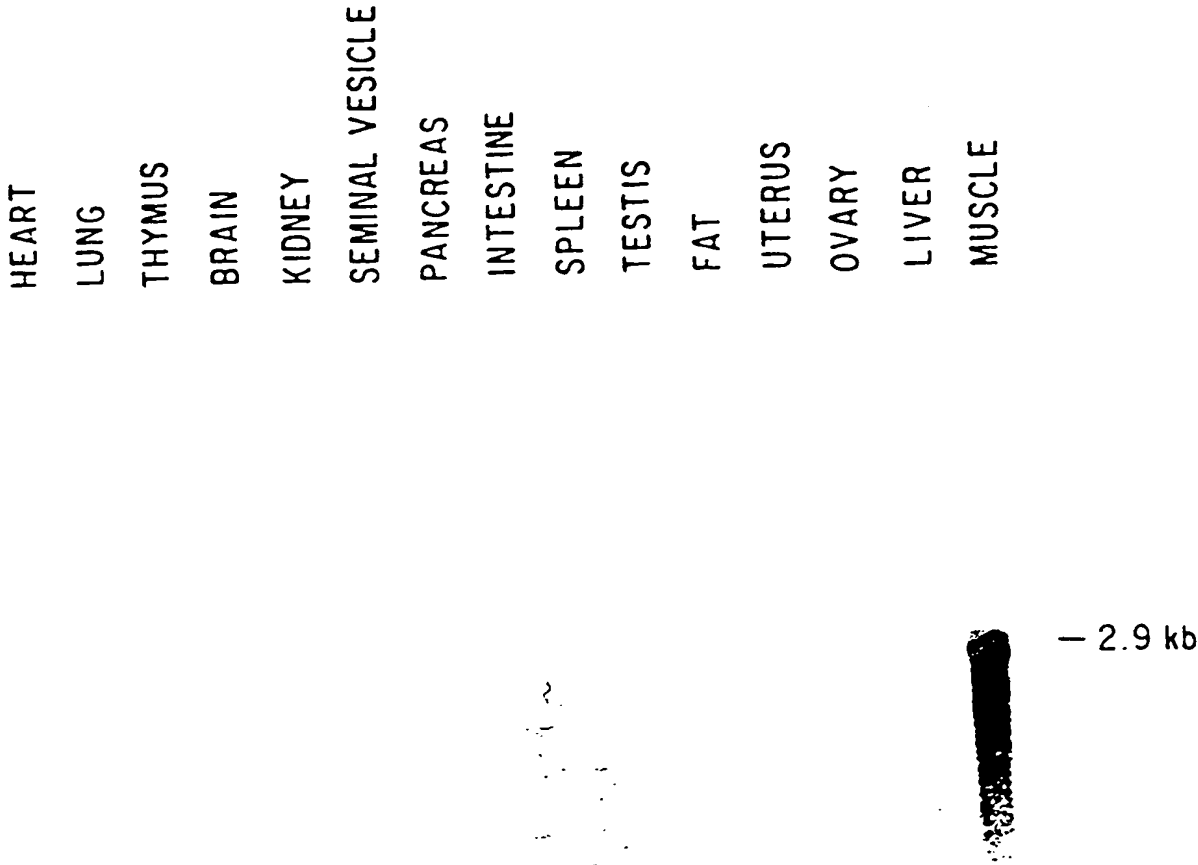


FIG.1a

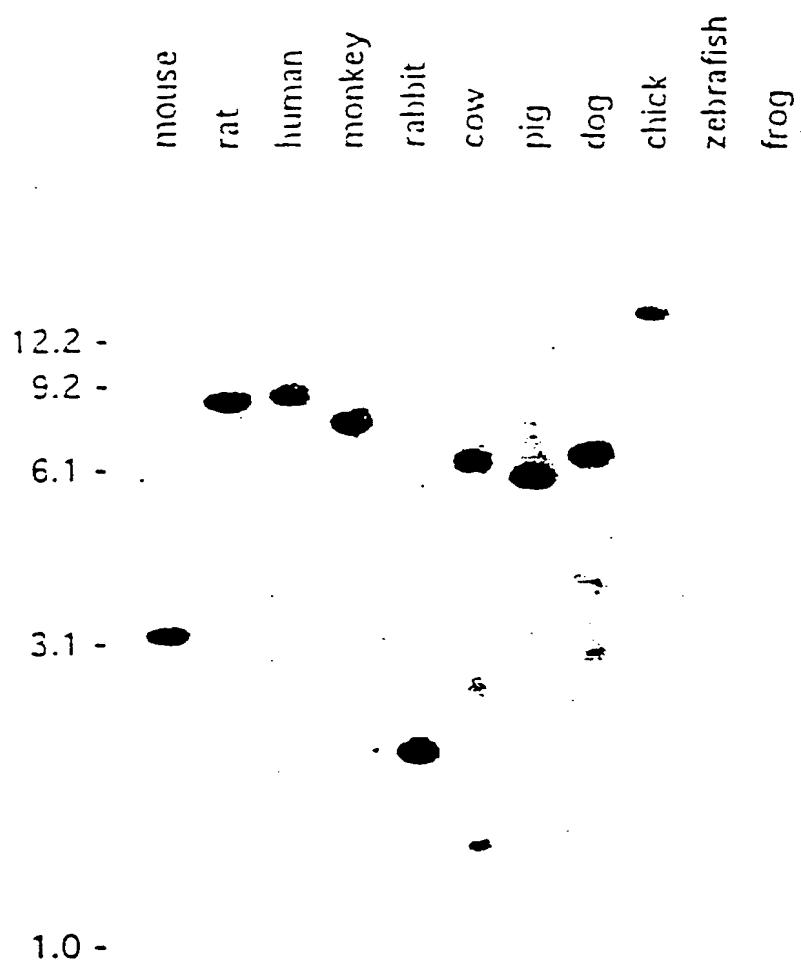


Figure 1b

FIG. 2a

FIG. 2b

GAA GAT GGG CTG AAT CCC TTT TTA GAA GTC AAA GTA ACA GAC ACA CCC AAG AGG TCC CGG
 E D G L N P F L E V K V T D T P K R S R
 AGA GAC TTT GGG CTT GAC TGT GAT GAA CAC TCC ACC GAA TCG CCG TGC TGT CGC TAC CCC
 R D F G L D C D E H S T E S R C C R Y P
 CTC ACC GTC GAT TTC GAA GCC TTT GGA TGG GAC TGG ATT ATT GCA CCC AAA AGA TAT AAG
 L T V D F E A F G W D W I I A P K R Y K
 GGT AAT TAC TGC TCT GGA GAG TGT GAA TTT GTG TTC TTA CAA AAA TAT CGG CAT ACT CAT
 A N Y C S G E C E F V F L Q K Y P H T H
 CTT GTG CAC CAA GCA AAC CCC AGA GGC TCG GCA GGC GGT TGC TGC ACC CCA ACA AAA ATG
 L V H Q A N P R G S A G P C C T P T K M
 TCT CCC ATT AAT ATG CTA TAT TTT AAT GGC AAA GAA CAA ATA ATA TAT GGG AAA ATT CCA
 S P I N M L Y F N G K E Q I I Y G K I P
 GCC ATG GTA GTA GAC CGG TGT GGG TGC TCG TGA GGT TTG CAT TAG CTT TAA AAT TTC CCA
 A M V V D R C G C S
 AAT CGT GGA AGG TCT TCC CCT CGA TTT CGA AAC TGT GAA TTT ATG TAC CAC AGG CTG TAG

Rat GDF-8

FIG. 2c

TTA GTA GTA AAG GCA CAA TTA TGG ATA TAC TTG AGG CAA GTC CAA AAA CCT ACA ACG GTG
 L V V K A Q L W I Y L R Q V Q K P T T V
 TTT GTG CAG ATC CTG AGA CTC ATT AAG CCC ATG AAA GAC GGT ACA AGA TAT ACT GGA ATT
 F V C I L R L I K P M K D G T R Y T G I
 GGA TGT TGG AAA CTT GAC ATG AAC CCA GGC ACT GGT ATC TGG CAG AGT ATT GAT GTG AAG
 G S L K L D M N P G T G I W C S I D V K
 ACA GTG CTG CAA AAT TGG CTC AAA CAG CCT GAA TCC AAT TTA GGC ATC GAA ATA AAA GGT
 T V L Q N W L K Q P E S N L G I E I K A
 TTT GAT GAG ACT GGA CGA GAT CTT GCT GTC ACA TTC CCA GGA CCG GGT GAA GAT GGA TTG
 F D E T G R D L A V T F P G P G E D G L
 AAC CCA TTT TTA GAG GTC AGA GTT ACA GAC ACA CCG AAA CCG TCC CCG AGA GAT TTT GGC
 N P F L E V R V T D T P K R S R R D F G
 CTT GAC TGT GAT GAG CAC TCA ACG GAA TCC CGA TGT TGT CCG TAC CCG CTG ACA GTG GAT
 L D C D E H S T E S R C C R Y P L T V D
 TTC GAA GGT TTT GGA TGG GAC TGG ATT ATA GCA CCT AAA AGA TAC AAA GCC AAT TAC TGC
 F E A F G W D W I I A P K R Y K A N Y C
 TCC GGA GAA TGC GAA TTT GTG TTT CTA CAG AAA TAC CCG CAC ACT CAC CTG GTA CAC CAA
 E G E C E F V F L Q K Y P H T H L V H Q
 GCA AAT CCC AGA GGC TCA GCA GGC CCT TGC TGC ACA CCC ACC AAG ATG TCC CCT ATA AAC
 A N P R G S A G P C C T P T K M S P I N
 ATG CTG TAT TTC AAT GGA AAA GAA CAA ATA ATA TAT GGA AAG ATA CCA GCC ATG GTT GTA
 M L Y F N G K E Q I I Y G K I P A M V V
 GAT CGT TGC GGG TGC TCA TGA GGC TGT CGT GAG ATC CAC CAT TCG ATA AAT TGT GGA AGC
 E R C G C S
 CAC CAA AAA AAA AAG CTA TAT CCC CTC ATC CAT CTT TGA AAC TGT GAA ATT ACG TAC GCT
 AGG CAT TGC C

Chicken GDF-8

FIG. 2d

GDF-8 SRRDFGLDCDEHSTESRCRYPLTVDF-EAFGWD-WIAPKRYKANYCSGECFVFLQKYP----
 GDF-1 RPRRDAEPVLGGPGGACRARRLYVSF-REVGWRWVAPRGFLANYCGGQCALPVALSGSGGPP
 BMP-2 REKRQAKHKQRKRLKSSCKRHPLVDF-SDVGWNDWI VAPPGYHAFYCHGECFPLADHLNS----
 BMP-4 KRSPKHHSQRARKKNKNCRRHSLVDF-SDVGWNDWI VAPPGYQAFYCHGDCPFPLADHLNS----
 Vgr-1 SRGSGSSDYNGSELKTACKKHELTVSF-CDLGWCDWIAPKGYAANYCDGECFPLNAHMNA----
 CP-1 LRMANVAENSSSDCRQACKKHELTVSF-RDLGWCDWIAPEGYAANYCEGECFPLNSYMNA----
 BMP-5 SRMSSVGDYNTSECKQACKKHELTVSF-RDLGWCDWIAPEGYAAFYCDGECFPLNAHMNA----
 BMP-3 EQTLKKARRKQWIEPRNCARRYLVDF-ADIGWSEWISPKSFDAYYCAGACFPMPKSLKPS----
 MIS GPGRAQRSAGATAADGPCALRELSVDL-----RAERSVLIPETYOANNCGVCGWQSDRNPFRY--
 Inhibin α ALRLLQRPPEEPAHANCHRVALLNLSF-CELGWERWIVYPPSFIFHYCHGCGGLHIPNLSLPV-
 Inhibin β A HRRRRRGLECDGKV-NICCKKQCFVSVF-KDIGWNDWIAPSGYHANYCEGECFSLIAGTSGSSL-
 Inhibin β B HRIRKRGLECDGRT-NLCCRCQCFIDF-RLIGWNDWIAPTGYGNYCEGSCPAYLAGVPGSAS-
 TGF- β 1 HRRALDTNYCFSTEKNCCVRQLYIDFRKDLGWK-WIHEPKGYHANFCLGPCPYIWSLD-----
 TGF- β 2 KKRALDAAYCFRNVCNCCLRPLYIDFRDLGWK-WIHEPKGYANFCAGACPYLWSSD-----
 TGF- β 3 KKRALDTNYCFRNLENCVVRPLYIDFRDLGWK-WVHEPKGYANFCAGACPYLRSAD-----

GDF-8 -HTHLVHQANPRG-----SAGPCCT-PTKMSPINMLYF-NGKEQIIYGKIPAMVVDRCGCS
 GDF-1 ALNHAVLRALMHA-AAPGAADLPCCV-PARLSPISVLFF-DNSDNVLRQYEDMVVDECCGR
 BMP-2 -TNHAIQTLVNS-VNSKIPKACCV-PTELSAISMLYL-DENEKVVLKNYQDMVVECCGR
 BMP-4 -TNHAIQTLVNS-VNSSIPKACCV-PTELSAISMLYL-DEYDKVVLKNYGEMVVECCGR
 Vgr-1 -TNHAIQTLVHL-MNPEYVPKPCA-PTKLNASVLYF-DDNSNVILKKYRNMVVRACGCH
 CP-1 -TNHAIQTLVHL-INPETVPKPCA-PTQLNASVLYF-DDSSNVILKKYRNMVVRACGCH
 BMP-5 -TNHAIQTLVHL-MFPDHVPKPCA-PTKLNASVLYF-DDSSNVILKKYRNMVVRACGCH
 BMP-3 -NHATIQSIVRA-VGVVPGIPEPCCV-PEKMSSLSILFF-DENKNVVLKVYPNMTVE SCACR
 MIS -GNHVLLLLKMQA-RGAALARPPCCV-PTAYAGKLLISLSEER-ISAHVPMNVATECCGR
 Inhibin α -PGAPPTPACPYSL-LLPGAQPCCAALPGTMRPLHVRTTSDGGYSFKYETVNNLLTCHCACI
 Inhibin β A -SFHSTVINHYRMRGHSFANLKSCCV-PTKLRPMSMLYF-DDGQNIKKDIGNMIVECCGS
 Inhibin β B -SFHTAVVNQYRMRGLNPGT-VNSCQI-PTKLSTMSMLYF-DDEYNIVKRDVPMNIVECCGA
 TGF- β 1 -TQYSKVLALYNO-HNPGASAPCCV-PQALEPLIVYY-VGRKPKV-EQLSNMIVRSCKCS
 TGF- β 2 -TCHSRVLSLYNT-INPEASAPCCV-SQDLEPLTILYY-IGKTPKI-EQLSNMIVKSCCKS
 TGF- β 3 -TTHSTVLGLYNT-LNPEASAPCCV-PQDLEPLTILYY-VGRTPKV-EQLSNMIVKSCCKS

FIG.3a

human	1	110KLOLC	UVIYLPFLI	VAQPVLDLNE	BEOK	ENVEKPEQLNAC	THRQNT	KSR	BRIZAIKIKIQILBKLRLRLE	TAPNHSKDV	IRO								
murine		1110KLOHY	UVIYLPFLI	VAQPVLDLNE	BEOK	ENVEKPEQLNAC	THRQNT	KSR	BRIZAIKIKIQILBKLRLRLE	TAPNHSKDV	IRO								
rat																			
chicken																			
human	81	LLPKAPPLREL	LDQYDVORD	SDQSL	EDDDYHAT	TETI	ITHTP	TESDPL	HQV		160								
murine		LLPKAPPLREL	LDQYDVORD	SDQSL	EDDDYHAT	TETI	ITHTP	TESDPL	HQV		160								
rat																			
chicken																			
human	161	LRPVETPTT	VPVQILRLIK	PHKDG	TRYTO	IRBLKLD	HNPTQ	IGI	QBSID	VKT	VLONWLKQ	PEBNLO	IEIKAL	DENQ	HD	LAV			
murine		LRPVETPTT	VPVQILRLIK	PHKDG	TRYTO	IRBLKLD	HNPTQ	IGI	QBSID	VKT	VLONWLKQ	PEBNLO	IEIKAL	DENQ	HD	LAV			
rat																			
chicken		LRQVOKP	TTTVPVQILRLIK	PHKDG	TRYTO	IRBLKLD	HNPTQ	IGI	QBSID	VKT	VLONWLKQ	PEBNLO	IEIKAL	DENQ	HD	LAV			
human	241	TFPOQ	QEDGLNP	PLEVK	VTDT	PKRSRR	DPQL	DCDEH	STESR	CCRYPL	TVDP	EA	PQWDH	IAP	KRYKAN	YCS	QCEP	VP	LO
murine		TFPOQ	QEDGLNP	PLEVK	VTDT	PKRSRR	DPQL	DCDEH	STESR	CCRYPL	TVDP	EA	PQWDH	IAP	KRYKAN	YCS	QCEP	VP	LO
rat																			
chicken		TFPOQ	QEDGLNP	PLEVK	VTDT	PKRSRR	DPQL	DCDEH	STESR	CCRYPL	TVDP	EA	PQWDH	IAP	KRYKAN	YCS	QCEP	VP	LO
human	321	KYPHT	HLVHQAN	PRQ	SAG	PCCT	PTKH	SPIN	HLVFN	GKEQ	IYQK	IPAH	VVDR	CC	CB				
murine		KYPHT	HLVHQAN	PRQ	SAG	PCCT	PTKH	SPIN	HLVFN	GKEQ	IYQK	IPAH	VVDR	CC	CB				
rat																			
chicken		KYPHT	HLVHQAN	PRQ	SAG	PCCT	PTKH	SPIN	HLVFN	GKEQ	IYQK	IPAH	VVDR	CC	CB				

FIG. 3b

GDF-1	100	33	50	46	44	48	35	27	42	43	46	47	46	42	34	23	37	35	33	32	33	TCF- β 3
GDF-2	-	100	42	47	51	48	31	32	52	51	55	52	55	34	20	20	32	25	26	28	30	TCF- β 2
GDF-3	-	-	100	49	49	46	41	33	53	50	53	50	50	42	22	25	42	41	36	31	32	TCF- β 1
GDF-5	-	-	-	100	86	80	37	33	57	57	51	51	52	47	27	24	40	37	33	34	37	Inhibin β B
GDF-6	-	-	-	-	100	80	38	34	57	56	53	53	54	46	26	27	43	39	35	36	38	Inhibin β A
GDF-7	-	-	-	-	-	100	37	33	57	57	52	53	52	46	25	26	41	36	36	35	38	MIS
GDF-8	-	-	-	-	-	-	100	27	41	38	45	42	42	38	31	26	38	42	34	37	37	BMP-3
GDF-9	-	-	-	-	-	-	-	100	33	34	31	30	31	29	21	27	30	31	23	25	25	BMP-5
BMP-2	-	-	-	-	-	-	-	-	100	92	61	60	61	48	27	22	42	42	35	34	36	Vgr-1
BMP-4	-	-	-	-	-	-	-	-	-	100	60	58	59	47	27	22	41	42	34	33	35	OP-1
Vgr-1	-	-	-	-	-	-	-	-	-	-	100	87	91	44	24	25	44	41	35	37	39	BMP-5
OP-1	-	-	-	-	-	-	-	-	-	-	-	100	88	42	27	24	43	42	34	38	38	BMP-3
BMP-5	-	-	-	-	-	-	-	-	-	-	-	-	100	43	24	24	43	37	34	35	36	MIS
BMP-3	-	-	-	-	-	-	-	-	-	-	-	-	-	100	30	29	36	37	32	32	32	Inhibin β B
MIS	-	-	-	-	-	-	-	-	-	-	-	-	-	-	100	18	24	25	28	23	25	Inhibin β A
Inhibin α	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	100	26	25	23	22	24	Inhibin β B
Inhibin β A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	100	63	41	37	36	TCF- β 1
Inhibin β B	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	100	35	34	37	TCF- β 2
TCF- β 1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	100	74	78	TCF- β 3
TCF- β 2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	100	82	
TCF- β 3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	100	

FIG.4

FIG. 5a

1261 AAGTCATGGAAGGTCTTCCCCTCAATTTGAAACTGTGAATTCAAGCACCACAGGCTGTA 1320
1321 GGCCTTGAGTATGCTCTAGTAACGTAAGCACAAGCTACAGTGTATGAACTAAAAGAGAGA 1380
1381 ATAGATGCAATGGTTGGCATTCAACCACCAAAATAAACCATACTATAGGATGTTGTATGA 1440
1441 TTTCCAGAGTTTTTGAAATAGATGGAGATCAAATTACATTTATGTCCATATATGTATATT 1500
1501 ACAACTACAATCTAGGCAAGGAAGTGAGAGCACATCTTGTGGTCTGCTGAGTTAGGAGGG 1560
1561 TATGATTAAGGTAAGTCTTATTTCTAACAGTTTCACTTAATATTTACAGAAGAATC 1620
1621 TATATGTAGCCTTTGTAAAGGTAGGATTGTTATCATTTAAAAACATCATGTACACTTAT 1680
1681 ATTTGTATTGTATACTTGGTAAGATAAAATCCACAAAGTAGGAATGGGGCCTCACATAC 1740
1741 ACATTGCCATTCTATTATAATTGGACAATCCACCACGGTGCTAATGCAGTGTCTGAATGG 1800
1801 CTCCTACTGGACCTCTCGATAGAACTCTACAAAGTACGAGTCTCTCTCTCCCTTCCAG 1860
1861 GTGCATCTCCACACACACAGCACTAAGTGTCAATGCATTTTCTTTAAGGAAAGAAGAT 1920
1921 CTTTTTTCTAGAGGTCACTTTTCAGTCAACTCTAGCACAGCGGGAGTGACTGCTGCATC 1980
1981 TAAAAGGCAGCCAAACAGTATTCATTTTTTAATCTAAATTTCAAATCACTGTCTGCCT 2040
2041 TTATCACATGGCAATTTTGTGGTAAATAATGGAAATGACTGGTTCTATCAATATTGTAT 2100
2101 AAAAGACTCTGAAACAATTACATTTATATAATATGTATACAATATTGTTTTGTAAATAAG 2160
2161 TGTCTCCTTTTATATTTACTTTGGTATATTTTACACTAATGAAATTTCAAATCATTAAA 2220
2221 GTACAAAGACATGTCATGTATCACAAAAAGGTGACTGCTTCTATTTTACAGTGAATTAG 2280
2281 CAGATTCAATAGTGGTCTTAAACTCTGTATGTTAAGATTAGAAGGTTATATTACAATCA 2340
2341 ATTTATGTATTTTTTACATTATCACTTATGGTTTCATGGTGGCTGTATCTATGAATGTG 2400
2401 GCTCCAGTCAAATTTCAATGCCCCACCATTTTAAAAATTACAAGCATTACTAAACATAC 2460
2461 CAACATGTATCTAAAGAAATACAAATATGGTATCTCAATAACAGCTACTTTTTTATTTTA 2520
2521 TAATTTGACAATGAATACATTTCTTTTATTTACTTCAGTTTATAAATTGGAACCTTTGTT 2580
2581 TATCAATGTATTGTACTCATAGCTAAATGAAATATTTCTTACATAAAAAATGTGTAGAA 2640
2641 ACTATAAATTAAGTGTTTTTACATTTTTTGAAGGC 2676

FIG.5b

1 AAGAAAAGTAAAAGGAAGAAACAAGAACAAGAAAAAGATTATATTGATTTTAAATCAT 60
 M
 61 GCAAAAAGTCAACTCTGTGTTTATATTTACCTGTTTATGCTGATTGTTGCTGGTCCAGT 120
 Q K L Q L C V Y I Y L F M L I V A G P V
 121 GGATCTAAATGAGAACAGTGAGCAAAAAGAAAATGTGGAAAAAGAGGGCTGTGTAATGC 180
 D L N E N S E Q K E N V E K E G L C N A
 181 ATGTACTTGGAGACAAAACACTAAATCTTCAAGAATAGAAGCCATTAAGATACAAATCCT 240
 C T W R Q N T K S S R I E A I K I Q I L
 241 CAGTAAACTTCGTCTGGAACAGCTCCTAACATCAGCAAAGATGTTATAAGACAACCTTT 300
 S K L R L E T A P **N I S** K D V I R Q L L
 301 ACCCAAAGCTCCTCCACTCCGGAAGTGAATGATCAGTATGATGTCCAGAGGGATGACAG 360
 P K A P P L R E L I D Q Y D V Q R D D S
 361 CAGCGATGGCTCTTTGGAAGATGACGATTATCAGCTACAACGAAACAATCATTACCAT 420
 S D G S L E D D D Y H A T T E T I I T M
 421 GCCTACAGAGTCTGATTTTCTAATGCAAGTGGATGGAAAACCCAAATGTTGCTTCTTTAA 480
 P T E S D F L M Q V D G K P K C C F F K
 481 ATTTAGCTCTAAATACAATAACAATAAAGTAGTAAAGGCCCAACTATCGATATATTTGAG 540
 F S S K I Q Y N K V V K A Q L W I Y L R
 541 ACCCGTCGAGACTCCTACAACAGTGTGTTGTGCAAATCCTGAGACTCATCAAACCTATGAA 600
 P V E T P T T V F V Q I L R L I K P M K
 601 AGACGGTACAAGTATACTGGAATCCGATCTCTGAAACTTGACATGAACCCAGGCACTGG 660
 D G T R Y T G I R S L K L D M N P G T G
 661 TATTTGGCAGAGCATTGATGTGAAGACAGTGTGCAAAATTGGCTCAAACAACCTGAATC 720
 I W Q S I D V K T V L Q N W L K Q P E S
 721 CAACTTAGGCATTGAAATAAAAGCTTTAGATGAGAATGGTCATGATCTTGCTGTAACCTT 780
 N L G I E I K A L D E N G H D L A V T F
 781 CCCAGGACCAGGAGAAGATGGGCTGAATCCGTTTTTAGAGGTCAAGGTAACAGACACACC 840
 P C P G E D G L N P F L E V K V T D T P
 841 AAAAAGATCCAGAAGGGATTTTGGTCTTGACTGTGATGAGCACTCAACAGAATCAGGATG 900
 K **R S R R** D F G L D C D E H S T E S R C
 901 CTGTCGTTACCCTCTAACTGTGGATTTTGAAGCTTTTGGATGGGATTGGATTATCGCTCC 960
 C R Y P L T V D F E A F G W D W I I A P
 961 TAAAAGATATAAGGCCAATTACTGCTCTGGAGAGTGTGAATTTGTATTTTACAAAAATA 1020
 K R Y K A N Y C S G E C E F V F L Q K Y
 1021 TCCTCACTCATCTGGTACACCAAGCAAACCCAGAGGTTGAGCAGGCCCTTGCTGTAC 1080
 P H T H L V H Q A N P R G S A G P C C T
 1081 TCCCACAAAGATGTCTCCAATTAATATGCTATATTTTAAATGGCAAAGAACAAATAATATA 1140
 P T K M S P I N M L Y F N G K E Q I I Y
 1141 TGGGAAAATTCCAGCGATGGTAGACCGCTGTGGGTGCTCATGAGATTTATATTAAGC 1200
 G K I P A M V V D R C G C S *

FIG.5c

1201 GTTCATAACTTCCTAAAACATGGAAGGTTTTCCCTCAACAATTTTGAAGCTGTGAAATT 1260
1261 AAGTACCACAGGCTATAGCCCTAGAGTATGCTACAGTCACITTAAGCATAAGCTACAGTAT 1320
1321 GTAAACTAAAAGGGGAATATATGCAATGGTTGGCATTAAACCATCCAAACAAATCATAC 1380
1381 AAGAAAGTTTTATGATTTCCAGAGTTTTTGAGCTAGAAGGAGATCAAATTACATTTATGT 1440
1441 TCCTATATATTACAACATCGGCGAGGAAATGAAAGCGATTCTCCTTGAGTTCTGATGAAT 1500
1501 TAAAGGAGTATGCTTTAAAGTCTATTTCTTTAAAGTTTGTAAATTTTACAGAAAAAT 1560
1561 CCACATACAGTATTGGTAAAATGCAGGATTGTTATATACCATCATTGGAATCATCCTTAA 1620
1621 ACACTTGAATTTATATTGTATGGTAGTATACTTGGTAAGATAAAATCCACAAAAATAGG 1680
1681 GATGGTGCAGCATATGCAATTTCCATTCTATTATAATTGACACAGTACATTAACAATCC 1740
1741 ATGCCAACGGTCTAATACGATAGGCTGAATGTCTGAGGCTACCAGGTTTATCACATAAA 1800
1801 AAACATTCAGTAAAATAGTAAGTTTCTCTTTCTTCAGGTGCATTTTCTACACCTCCAA 1860
1861 ATGAGGAATCGATTTTCTTTAATGTAAGAAGAATCATTTTTCTAGAGGTTGCCTTTCAAT 1920
1921 TCTGTAGCATACTTGGAGAACTGCATTATCTTAAAAGGCAGTCAAATGGTGTTTGT TTT 1980
1981 TATCAAAATGTCAAAATAACATACTTGGAGAAGTATGTAATTTTGTCTTTGGAAAAATAC 2040
2041 AACACTGCCCTTTGCAACACTGCAGTTTTTATCGTAAAATAATAGAAATGATCGACTCTAT 2100
2101 CAATATTGTATAAAAAGACTGAAACAATGCATTTATATAATATGTATACAATATTGTTTT 2160
2161 GTAAATAAGTGCTCTCTTTTTTATTTACTTTGGTATATTTTTTACACTAAGGACATTTCAA 2220
2221 ATTAAGTACTAAGGCACAAAGACATGTCATGCATCACAGAAAAGCACTACTTATATTTCT 2280
2281 AGAGCAAATTAGCAGATTAAATAGTGGTCTTAAAACCTCCATATGTTAATGATTAGATGGT 2340
2341 TATATTACAATCATTTTTATTTTTTTTACATGATTAACATTCACTTATGGATTCATGATG 2400
2401 GCTGTATAAAGTGAATTTGAAATTTCAATGGTTTACTGTCAATTGTGTTTAAATCTCAACG 2460
2461 TTCCATTATTTTAATACTTGCAAAACATTACTAAGTATACCAAAATAATTGACTCTATT 2520
2521 ATCTGAAATGAAGAATAAACTGATGCTATCTCAACAATAACTGTTACTTTTATTTTATAA 2580
2581 TTTGATAATGAATATATTTCTGCATTTATTTACTTCTGTTTTGTAAATTGGGATTTTGT 2640
2641 AATCAAATTTATTGTACTATGACTAAATGAAATTTTCTTACATCTAATTTGTAGAAAC 2700
2701 AGTATAAGTTATATTAAAGTGTTTTTACATTTTTTTGAAAGAC 2743

FIG.5d

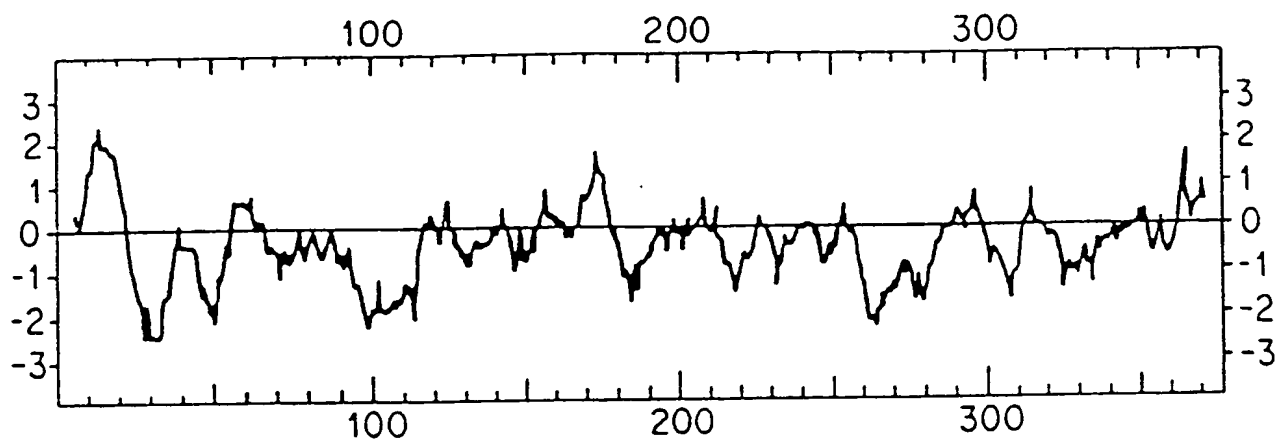


FIG. 6a

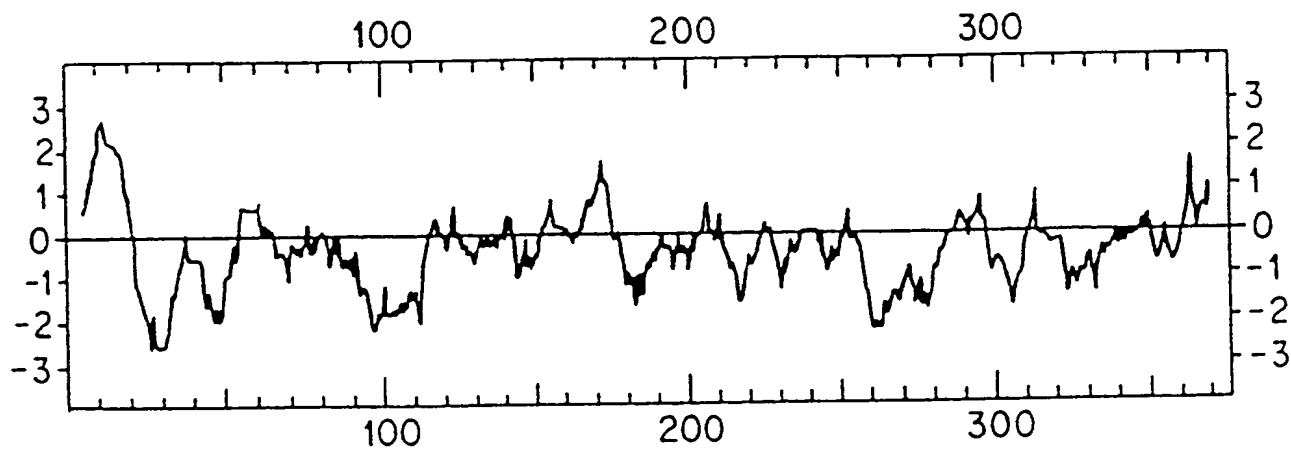


FIG. 6b

```

1  MMQKLQMYVYIYLFMLIAAGPVDLNEGSEREENVEKEGLCNACAWRONTR  50
   ||||| ||||| ||||| || ||||| ||||| |||||
1  MMQKLQLCVYIYLFMLIVAGPVDLNESEQKENVEKEGLCNACTWRQNTK  49

51  YSRIEAIKIQILSKLRLETAPNISKDAIRQLLPRAPPLRELIDQYDVQRD  100
   ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
50  SSRIEAIKIQILSKLRLETAPNISKDVIRQLLPKAPPLRELIDQYDVQRD  99

101 DSSDGSLEDDDYHATTETITMPTESDFLMQADGKPKCCFFKFSSKIQYN  150
   ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
100 DSSDGSLEDDDYHATTETITMPTESDFLMQVDGKPKCCFFKFSSKIQYN  149

151 KVVKAQLWIYLRPVKTPPTVFVQILRLIKPMKDCTRYTGIRSLKLDMSPG  200
   ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
150 KVVKAQLWIYLRPVEPTTFVFVQILRLIKPMKDCTRYTGIRSLKLDMPG  199

201 TGIWQSIDVKTVLQNWLKQPE SNLGIEIKALDENGHD LAVTFPGPGEDGL  250
   ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
200 TGIWQSIDVKTVLQNWLKQPE SNLGIEIKALDENGHD LAVTFPGPGEDGL  249

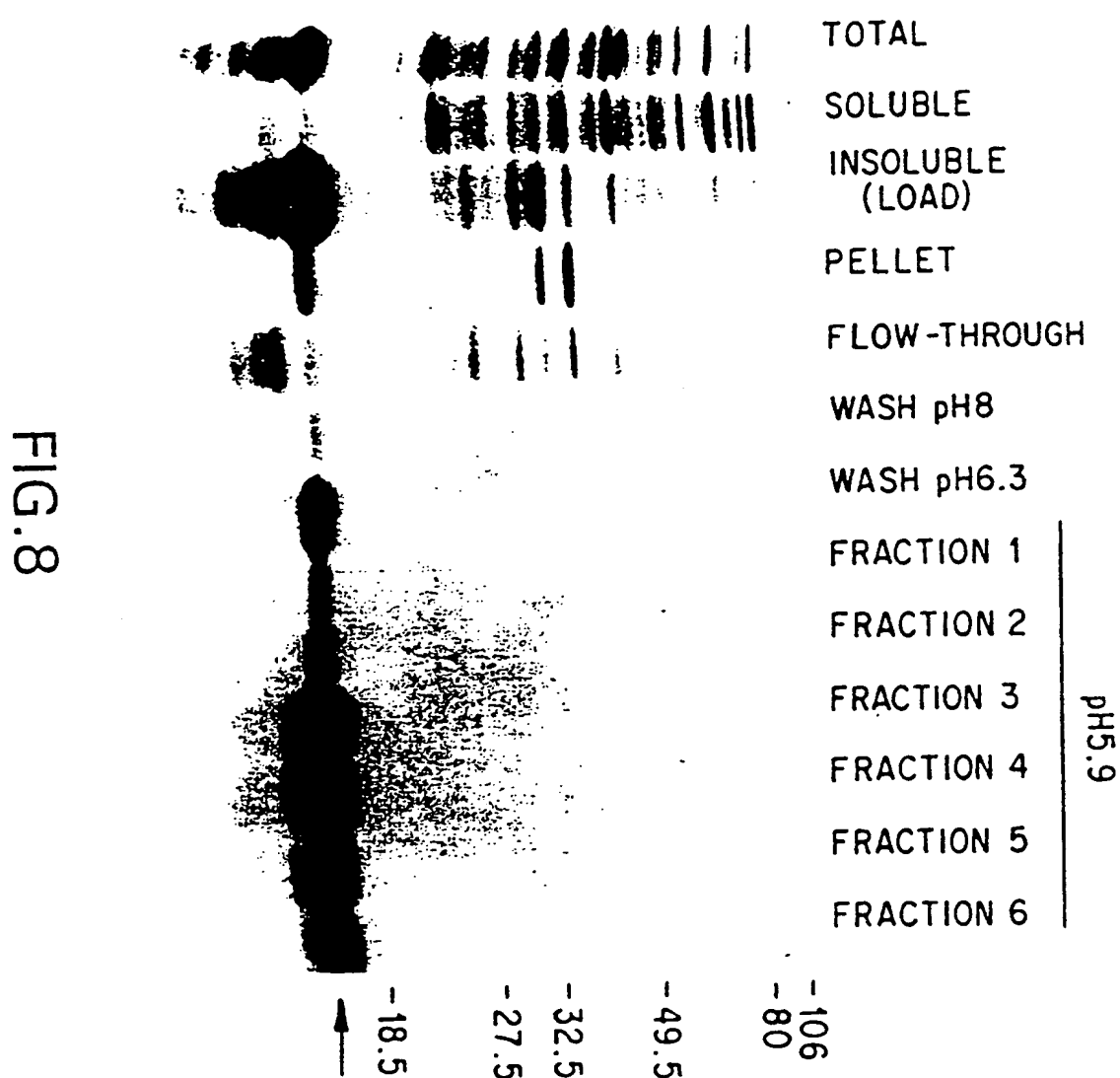
251 NPFLEVKVTDTPKRSRRDFGLDCDEHSTESRCCRYPLTVDFEAFGWOWII  300
   ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
250 NPFLEVKVTDTPKRSRRDFGLDCDEHSTESRCCRYPLTVDFEAFGWOWII  299

301 APKRYKANYCSGECEVFVLQKYPHTL VHQANPRGSAGPCCTPTKMSPIN  350
   ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
300 APKRYKANYCSGECEVFVLQKYPHTL VHQANPRGSAGPCCTPTKMSPIN  349

351 MLYFNGKEQIYGKIPAMVVDRCCGS  376
   ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
350 MLYFNGKEQIYGKIPAMVVDRCCGS  375

```

FIG.7



ANTISENSE SENSE

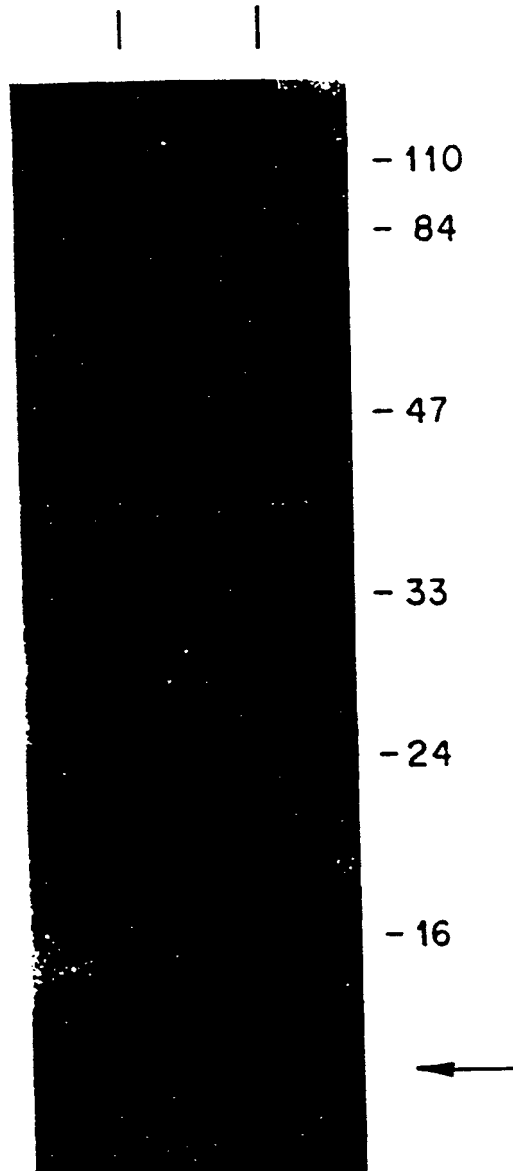


FIG. 9

FIG. 10A

HEART
LUNG
THYMUS
BRAIN
KIDNEY
SEMINAL VESICLE
PANCREAS
INTESTINE
SPLEEN
TESTIS
MUSCLE
LIVER
OVARY
FAT
UTERUS

—2.9 kb

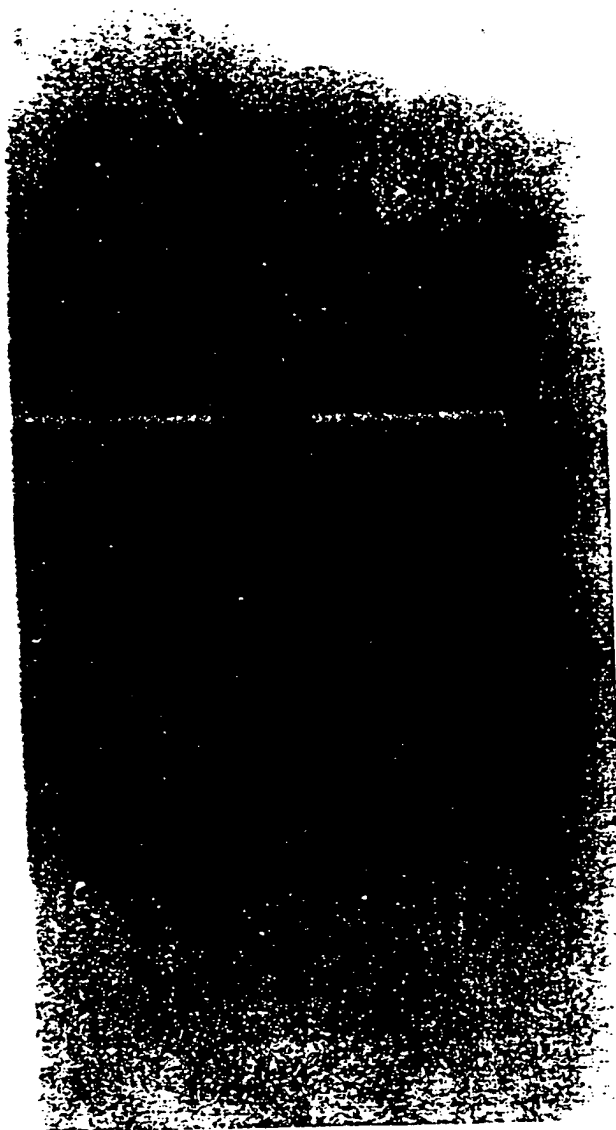
12.5 d PLACENTA

14.5 d PLACENTA

16.5 d PLACENTA

12.5 d EMBRYO

18.5 d EMBRYO



-2.9 kb

FIG. 10b

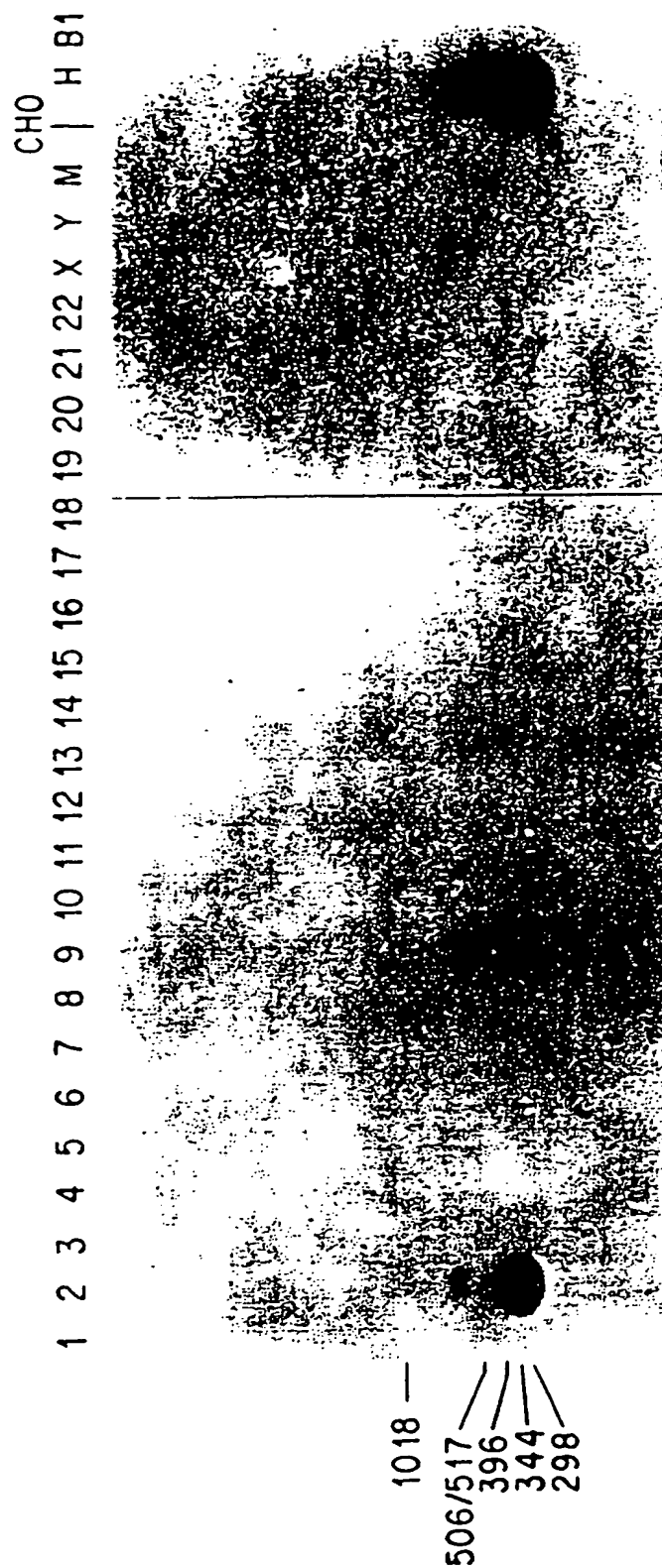


FIG.11

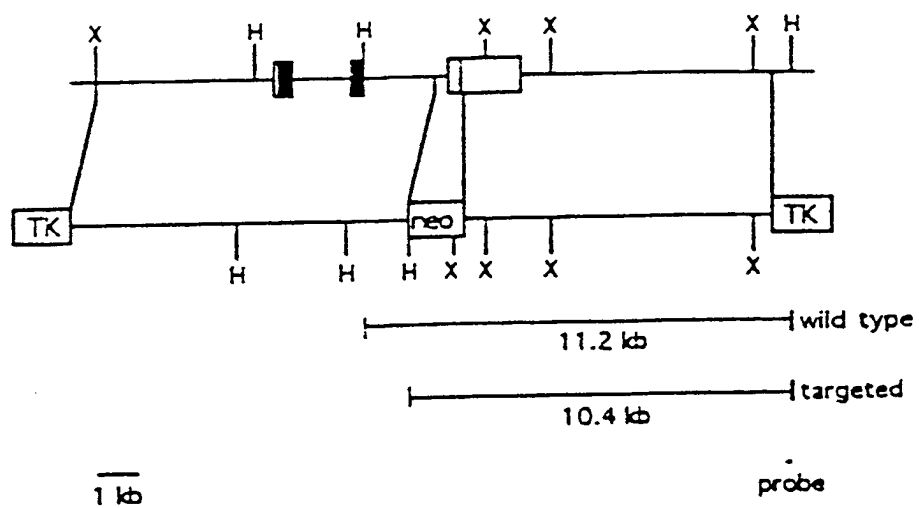


Figure 12a

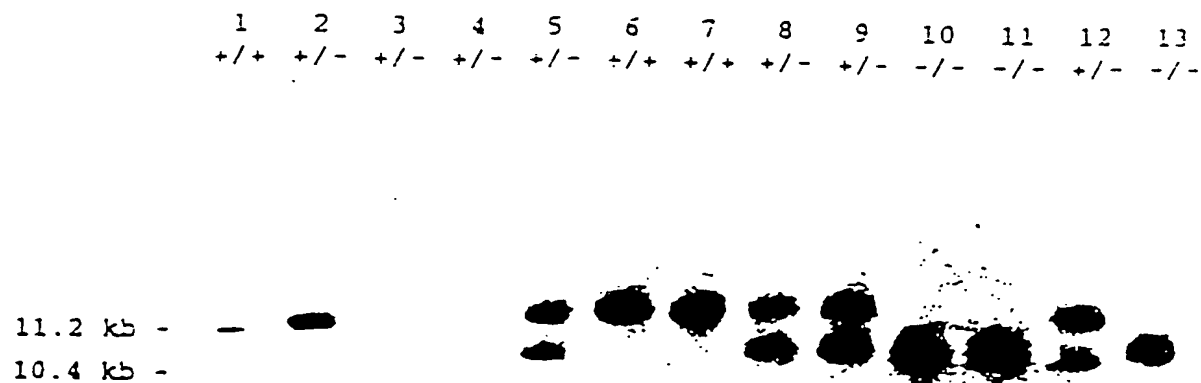


Figure 12b

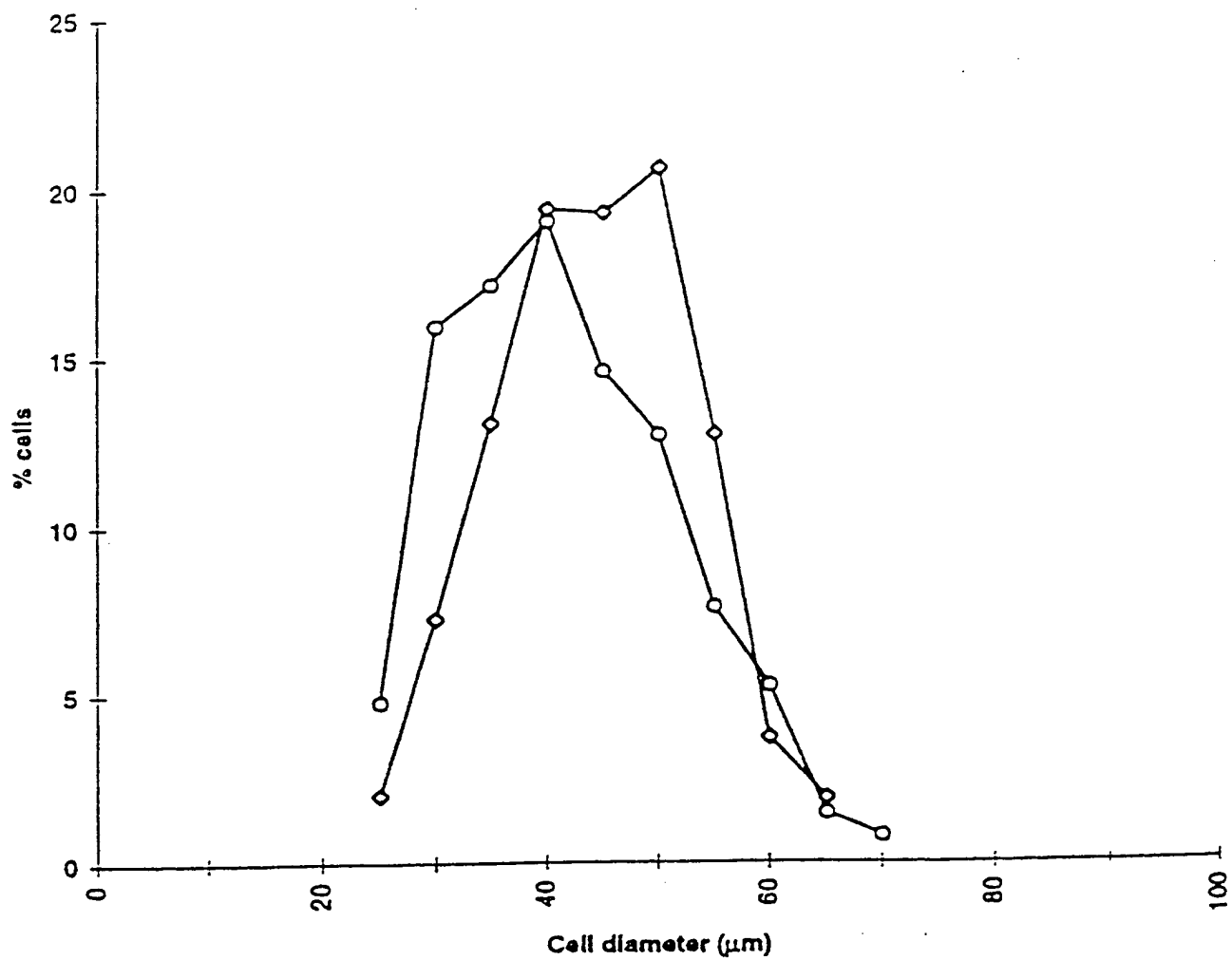


Figure 3a
bottom

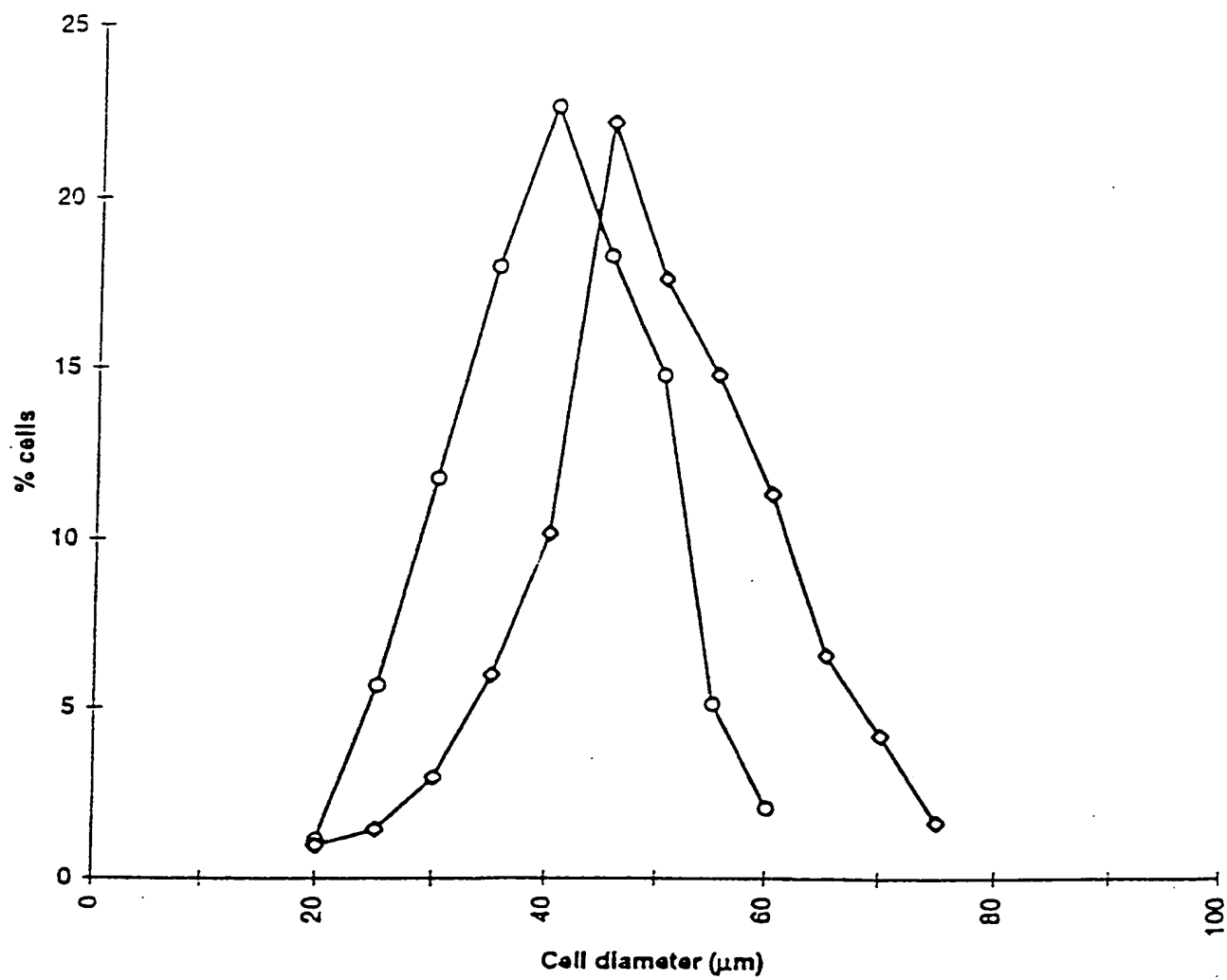


Figure 13b
bottom

FIGURE 14a

```

1/1                               31/11
ATG CAA AAA CTC CAA CTC TGT GTC TAT ATT TAC CTC TTT ATG CTC ATT GTT GGT GGT CCA
M Q K L C L C V Y I Y L F M L I V A G P
61/21                               91/31
GTC GAT CTA AAT CAG AAC AGT GAG CAA AAA GAA AAT CTC GAA AAA GAG GGG CTC TGT AAT
V D L N E N S E Q K E N V E K E G L C N
121/41                               151/51
GCA TGT ACT TGG ACA CAA AAC ACT AAA TGT TCA AGA ATA GAA GGC ATT AAA ATA CAA ATC
A C T W R C N T K S S R I E A I K I Q I
181/61                               211/71
CTC ACT AAA CTT GGT CTC GAA ACA GGT GGT AAC ATC AGC AAA GAT GGT ATA ACA CAA CTT
L S X L A L E T A P N I S K D A I R Q L
241/81                               271/91
TTA CCC AAA GGT GGT CCA CTC CGG GAA CTC ATT GAT CAG TAT GAT GTC CAG AGG GAT GAC
L P K A P P L R E L I D Q Y D V Q R D D
301/101                               331/111
AGC AGC GAT GGC TGT TTT GAA GAT GAC GAT TAT CAG GGT ACA AGC GAA ACA ATT ATT AGC
S S D G S L E D D D Y H A T T E T I I T
361/121                               391/131
ATG CTT ACA GAG TGT GAT TTT TTA ATG CAA GTG GAT GGA AAA CCC AAA TGT TGG TTT TTT
M P T E S D F L M Q V D G K P K C C F F
421/141                               451/151
AAA TTT AGC TGT AAA ATA CAA TAC AAT AAA CTC GTA AAG GGC CAA CTA TGG ATA TAT TTT
K F S S K I Q Y N K V V K A Q L W I Y L
481/161                               511/171
ACA GGC GTC CAG ACT GGT ACA ACA CTC TTT GTC CAA ATC CTC ACA CTC ATC AAA CTT ATG
R P V E T P T T V F V Q I L R L I K P M
541/181                               571/191
AAA GAC GGT ACA AGG TAT ACT GGA ATC CCA TGT CTC AAA CTT GAC ATG AAC CCA GGC ACT
K D G T R Y T G I R S L K L D M N P G T
601/201                               631/211
CTT ATT TGG CAG AGC ATT GAT GTC AAG ACA GTC TTT CAA AAT TGG CTC AAA CAA CTT GAA
G I W Q S I D V K T V L Q N W L K Q P E
661/221                               691/231
TGC AAC TTA GGC ATT GAA ATA AAA GGT TTA GAT GAG AAT GGT CAT GAT CTT GGT GTA ACC
S N L G I E I K A L D E N G H D L A V T
721/241                               751/251
TTG CCA GGA CCA GGA GAA GAT GGG CTC AAT CCC TTT TTA GAG GTC AAG GTA ACA GAC ACA
F P G P G E D G L N P F L E V K V T D T
781/261                               811/271
CCA AAA ACA TGG ACA AGG GAT TGT GGT CTT GAC TGT GAT GAG CAC TCA ACA GAA TGG CCA
P K R S R R D F G L D C D E H S T E S R
841/281                               871/291
TGC TGT GGT TAC CCG CTA ACT GTC GAT TTT GAA GGT CTT GCA TGG GAT TGG ATT ATC GGT
C C R Y P L T V D F E A L G W D W I I A
901/301                               931/311
GCT AAA ACA TAT AAG GGC AAT TAC TGG TGT GGA GAG TGT GAA TTT GTA TTT TTA CAA AAA
P K R Y K A N Y C S G E C E F V F L Q K
961/321                               991/331
TAT GGT CAT ACT CAT CTC GTA CAC CAA CCA AAC CCC ACA GGT TCA CCA GGC CTT TGG TGT
Y P K T H L V H C A N P R C S A G P C C
1021/341                               1051/351
ACT GGC ACA AAG ATG TGT CCA ATT AAT ATG CTA TAT TTT AAT GGC AAA GAA CAA ATA ATA
T P T K M S P I N M L Y F N G K E C I I
1081/361                               1111/371
TAT GGC AAA ATT CCA GGC ATG GTA GTA GAC CCG TGG GGC TGG TCA TCA
Y C K I P A M V V D R C C C S

```

Baboon GDF-8

FIGURE 14b

```

1/1                               31/11
ATG CAA AAA CTG CAA ATG TGT GTT TAT ATT TAC CTA TTT ATG CTG ATT GTT GGT GCG CCA
M Q K L C I S V Y I Y L F H L I V A G P
61/21                               91/31
CTG GAT CTG AAT GAG AAC AGC GAG CAG AAC GAA AAT CTG GAA AAA GAG GCG CTG TGT AAT
V D L N E N S E Q K E N V E K E G L C N
121/41                               151/51
GCA TGT TTT TGG AGG GAA AAC ACT ACA TCC TCA AGA CTA GAA GCG ATA AAA ATC CAA ATC
A C L W R E N T T S S R L E A I K I Q I
181/61                               211/71
CTG AGT AAA CTT GCG CTG GAA ACA GGT GGT AAC ATC AGC AAA GAT GGT ATC AGA CAA CTT
L S K L R L E T A P N I S K D A I R Q L
241/81                               271/91
TTG GCG AAG GGT GGT CCA CTC CTG GAA CTG ATT GAT CAG TTC GAT GTC CAG AGA GAT GCG
L P K A P P L L E L I D Q F D V Q R D A
301/101                               331/111
AGC AGT GAC GCG TCC TTG GAA GAC GAT GAC TAC CAC GCG AGG AGC GAA AGC GTC ATT ACC
S S D G S L E D D D Y H A R T E T V I T
361/121                               391/131
ATG CCG ACC GAG TGT GAT CTT CTA AGC CAA GTC GAA GGA AAA CCG AAA TGT TCG TTC TTT
M P T E S D L L T Q V E G K P K C C F F
421/141                               451/151
AAA TTT AGC TGT AAG ATA CAA TAC AAT AAA CTA CTA AAG GCG CAA CTC TCG ATA TAT CTG
K F S S K I Q Y N K L V K A Q L W I Y L
481/161                               511/171
AGG GGT GTC AAG ACT GGT GCG ACA GTG TTT GTC CAA ATC CTG AGA CTC ATC AAA CCG ATG
A P W H T P A T V F V C I L R L I K P M
541/181                               571/191
AAA GAC GGT ACA AGG TAT ACT GGA ATC CCA TCT CTC AAA CTT GAC ATG AAC CCA GCG ACT
K D G T R Y T G I R S L K L D M N P G T
601/201                               631/211
GGT ATT TCG CAG AGC ATT GAT GTG AAG ACA GTG TTG CAG AAC TCG CTC AAA CCA GGT GAA
G I W Q S I D V K T V L Q N W L K Q P E
661/221                               691/231
TCC AAG TTA GCG ATT GAA ATC AAA GGT TTA GAT GAG AAT GCG CAT GAT CTT GGT GTA ACC
S N L G I E I K A L D E N G H D L A V T
721/241                               751/251
TTG CCA GAA CCA GGA GAA GAT GGA CTG ATT CTT TTT TTA GAA GTC AAG GTA ACA GAC ACA
F P E P G E D G L T P F L E V K V T D T
781/261                               811/271
CCA AAA ACA TGT AGG ACA GAT TTT GCG CTT GAT TGT GAT GAA CAC TCG ACA GAA TGT CCA
P K R S R R D F G L D C D E H S T E S R
841/281                               871/291
TCC TGT GGT TAC GGT CTA ACT GTG GAT TTT GAA GGT TTT GGA TCG GAT TCG ATT ATT GCA
C C R Y P L T V D F E A F G W D W I I A
901/301                               931/311
GCT AAA ACA TAT AAG GCG AAT TAC TCG TGT GGA GAA TGT GAA TTT GTA TTT TTG CAA AAG
P K R Y K A N Y C S G E C E F V F L Q K
961/321                               991/331
TAT GGT CAT ACC CAT CTT GTG CAC CAA CCA AAC CCG AGA GGT TCA GCG GCG GCG TCG TGT
Y P H T H L V H Q A N P R G S A G P C C
1021/341                               1051/351
ACT GGT ACA AAG ATG TTT CCA ATT AAT ATG CTA TAT TTT AAT GCG CAA GGA CAA ATA ATA
T P T K M S P I N M L Y F N G E C G I I
1081/361                               1111/371
TAC GCG AAG ATT CCA GCG ATG CTA GTA GAT CCG TGT GCG TGT TCA TCA
Y G K I P A M V V D R C G C S

```

Bovine GDF-8

FIGURE 14c

```

1/1
ATG CAA AAG CTA GCA GTC TAT GTT TAT ATT TAC CTG TTC ATG CAG ATC GCG GTT CAT CCG
M Q K L A V Y V Y I Y L F M Q I A V D P

61/21
CTG GGT CTG GAT GCG AGT AGT CAG CCG ACA GAG AAC GGT CAA AAA GAG GGA CTG TGC AAT
V A L D G S S Q P T E N A E K D G L C N

121/41
GCT TGT ACC TGG AGA CAG AAT ACA AAA TCC TCC AGA ATA GAA GCG ATA AAA ATT CAA ATC
A C T W R Q N T K S S R I E A I K I Q I

181/61
CTC ACC AAA CTG GCG CTG GAA CAA GCA CCT AAC ATT AGC AGC GAG GTT ATT AAG CAG CTT
L S K L R L E Q A P N I S R D V I K Q L

241/81
TTA CCG AAA GGT GGT CCA CTG CAG GAA CTG ATT GAT CAG TAT CAT GTC CAG AGG GAG GAG
L P K A F P L Q E L I D Q Y D V Q R D D

301/101
AGT AGC GAT GCG TGT TTT GAA GAC GAT GAC TAT CAT GCG ACA ACC GAG AGC ATT ATC ACA
S S D G S L E D D D Y H A T T E T I I T

361/121
ATG CGT ACC GAG TGT GAT TTT CTT GTA CAA ATG GAG GGA AAA CCA AAA TGT TGG TTT TTT
M P T E S D F L V Q H E G K P K C C F F

421/141
AAG TTT ACC TGT AAA ATA CAA TAT AAC AAA GTA GTA AAG GCA CAA TTA TGG ATA TAC TTG
K F S S K I Q Y N K V V K A Q L W I Y L

481/161
AGC CAA CTG CAA AAA CTT ACA AGC GTC TTT GTC CAG ATT CTS AGA CTS ATT AAG GCG ATG
E C V C K F T T V F V C I L R L I K F M

541/181
AAA GAC GGT ACA AGA TAT ACT GCA ATT CGA TGT TTT AAA GTT GAC ATG AAC CCA GCG ACT
K D G T R Y T G I R S L K L D M N P G T

601/201
GCT ATT TGG CAG AGT ATT GAT GTC AAG ACA GTC CTG CAA AAT TGG CTC AAA CAG CTT GAA
G I W Q S I D V K T V L Q N W L K Q P E

661/221
TGG AAT TTA GCG ACC GAA ATA AAA GGT TTT CAT CAG ACT GCA CCA GAT CTT GGT GTC ACA
S N L G I E I K A F D E T G R D L A V T

721/241
TTG CCA GCA CCG GGT GAA GAT GCA TTT AAC CCA TTT TTA GAG GTC AGA GTT ACA GAG ACA
F P G P G E D G L N P F L E V R V T D T

781/261
CGG AAA CCG TTT CCG AGA GAT TTT GCG CTT GAC TGT CAT CAG CAT TCA AGC GAA TCC CGA
P K R S R R D F G L D C D E H S T E S R

841/281
TGT TGT CCG TAC CCG CTG ACA GTG GAT TTT GAA GGT TTT GCA TGG GAC TGG ATT ATA GCA
C C R Y P L T V D F E A F G W D W I I A

901/301
CCT AAA AGA TAC AAA GCG AAT TAC TCG TCG GGA GAA TCG CAA TTT GTC TTT CTA CAG AAA
P K R Y K A N Y C S G E C E F V F L Q K

961/321
TAC CCG CAC ACT CAC CTG GTA CAG CAA GCA AAT CCG AGA GCG TCA GCA GCG CTT TCG TCG
Y P H T H L V H Q A N P R C S A G P C C

1021/341
ACA CCG ACC AAG ATG TCG CCG ATA AAC ATG CTG TAT TTT AAT GCA AAA GAA CAA ATA ATA
T P T K M S P I N M L Y F N G K E Q I I

1081/361
TAT GCA AAG ATA GCA CCG ATG CTT GTA CAT CCG TCG GCG TCG TCA TCA
Y G K I P A M V V D R C G C S

```

Chicken GDF-8

FIGURE 14d

```

1/1                               31/11
ATG ATT CAA AAA CCG CAA ATG TAT GTT TAT ATT TAC CTC TTT GTC CTC ATT GGT GGT GCG
M I Q K P Q M Y V Y I Y L F V L I A A G
61/21                               91/31
CCA CTC CAT CTA AAT CAG GAC AGT CAG ACA GAG CGG AAT GTC CAA AAA GAG CGG CTC TGT
P V D L N E D S E R E A N V E K E G L C
111/41                               151/51
AAT CGG TGT CCG TCG ACA CAA AAC ACA AGG TAC TCG ACA ATA CAA CGC ATA AAA ATT CAA
N A C A W R Q N T P Y S R I E A I K I Q
191/61                               211/71
ATG CTC AGT AAA CTC CCG CTC GAA ACA GCG CTT AAC ATC AGC AAA GAT GGT ATA ACA CAA
I L S K L R L E T A P N I S K D A I R Q
241/81                               271/91
CTT CTC CCG ACA CGG CTT CCA CTC CCG GAA CTC ATC CAT CAG TAC GAC CTC CAG AGG CAT
L L P R A P P L R E L I D Q Y D V Q R D
301/101                               331/111
GAC AGC AGT CAG CGG TTT TTT GAA CAT GAC CAT TAT CAC GGT ACC AGC GAA ACA ATC ATT
D S S D G S L E D D D Y H A T T E T I I
361/121                               391/131
ACC ATG CTT ACC CAG TTT GAC TTT CTA ATG CAA GCG CAT GGA AAG CGC AAA TGT TCG TTT
T M P T E S D F L M Q A D G K P K C C F
421/141                               451/151
TTT AAA TTT ACC TTT AAA ATA CAG TAC AAC AAA GTC GTA AAG GCG CAG CTC TCG ATA TAT
F K P S S K I Q Y N K V V K A Q L W I Y
481/161                               511/171
CTC ACA CGG CTC AAG ACT CTT ACA ACA CTC TTT CTC CAA ATC CTC ACA CTC ATC AAA CGC
L R A V K T P T T V F V Q I L R L I K P
541/181                               571/191
ATG AAA GAC CTT ACA AGG TAT ACC GGA ATC CCA TGT CTC AAA CTT GAC ATG ACC CCA GCG
M K D G T R Y T G I R S L K L D M S P G
601/201                               631/211
ACT GGT ATT TCG CAG AGT ATT CAT CTC AAG ACA GTC TTT CAA AAT TCG CTC AAA CAG CTT
T G I W Q S I D V K T V L Q N W L K Q P
661/221                               691/231
GAA TCG AAC TTA CGC ATT CAA ATT AAA CTT TTT CAT GAG AAT GCG CAT CAT CTT GGT GTA
E S N L G I E I K A L D E N G H D L A V
721/241                               751/251
ACC TTT CCA GGA CCA GGA CAA CAT CGG CTC AAT CGG TTT TTA CAA GTC AAA GTA ACA GAC
T F P G P G E D G L N F F L E V K V T D
781/261                               811/271
ACA CGG AAG ACC TCG CCG ACA GAC TTT CGG CTT GAC TGT CAT GAA CAC TCG ACC GAA TCG
T P K R S R R D F G L D C D E H S T E S
841/281                               871/291
CGG TCG TGT CCG TAC CCG CTC ACC GTC CAT TTT GAA CGG TTT GGA TCG GAC TCG ATT ATT
R C C R Y P L T V D F E A F G W D W I I
901/301                               931/311
GCA CGG AAA ACA TAT AAG CTT AAT TAC TCG TTT GCA CAG TGT CAA TTT CTC TTT TTA CAA
A P K R Y K A N Y C S G E C E F V F L Q
961/321                               991/331
AAA TAT CCG CAT ACT CAT CTT CTC CAC CAA GCA AAC CGG ACA GCG TCG GCA GCG CTT TCG
K Y P H T H L V H C A N P P C S A C P C
1021/341                               1051/351
TCC ACC CCA ACA AAA ATG TGT CCG ATT AAT ATG CTA TAT TTT AAT GCG AAA GAA CAA ATA
C T P T K M S P I N M L Y F N G K E C I
1081/361                               1111/371
ATA TAT CCG AAA ATT CCA CGG ATG CTA GTA CAC CGG TGT CCG TCG TCG TCA
I Y G K I P A H V V D R C G C S

```

Rat GDF-8

FIGURE 14c

1/1
 ATG CAA AAG CTA GGA CTC TAT GTT TAT ATT TAC CTC TTC ATG CAG ATT TTA CTC CAT CCG
 H C K L A V Y V Y I Y L P M Q I L V H P
 31/11
 61/21
 CTC CTT CTT CAT CCG AAT AAT CAG CCG ACA CAG AAC CTT CAA AAA CAG GGA CTC TCG AAT
 V A L E G S S Q P T E N A E K D G L C N
 91/31
 121/41
 GCT TCG ACC TCG AGA CAG AAT AAT AAA TCG TCG AGA ATA CAA CCG ATA AAA ATT CAA ATC
 A C T W R C N T R S S R E A I K C G C
 151/51
 181/61
 CTC ACC AAA CTC CCG CTC CAA CAA CCA CTT AAC ATT AGC AGC CAG CTC ATT AAA CAA CTC
 L S K L R L E Q A P N I S R D V I R Q L
 211/71
 241/81
 TTA CCG AAA CTT CTT CCG CTC CAG CAA CTC ATT CAT CAG TAT CAG CTC CAG AGA CAG GAG
 L P K A P P L G E L I E G Y D V G R D D
 271/91
 301/101
 AAT ACC CAT CCG TCG TCG CAA GAG GAT GAG TAT CAT CCG ACA ACC CAA ACC ATT ATC ACA
 S S D G S L E D D D Y H A T T E T I C T
 331/111
 361/121
 ATG CTT ACC CAG TCG CAT TTT CTC CTA CAA ATG CAG CCA AAA CCA AAA TCG TCG TCG TCG
 M P T E S D P L V Q M E G K P K C C F F
 391/131
 421/141
 AAC TCG ACC TCG AAA ACA CAA TAT AAC AAA CTA CTA AAC CCA CAA TTA TCG ATA TAC TCG
 K F S S K C G Y N K V V K A Q L W E Y L
 451/151
 481/161
 ACC CAA CTC CAA AAA CTT ACA ACC CTC TTT CTC CAG ATC CTC AGA CTC ATT AAA CCG ATC
 F C T R S P T T V F V Q C L R L C K P M
 511/171
 541/181
 AAA CAG CTT ACA AGA TAT AAT CCA ATT CCA TCG TCG AAA CTC CAG ATG AAC CCA CCG AAT
 K D C T R Y T G C R S L K L D M N P C T
 571/191
 601/201
 GCT ATC TCG CAG AAT ATT CAT CTC AAC ACA CTC TCG CAA AAT TCG CTC AAA CAG CTT CAA
 G E W G S C D V K T V L Q N W L K Q P E
 631/211
 661/221
 TCG AAT TTA CCG AAT CAA ATA AAA CTT TTT CAT CAG AAT CCA CCA CAT CTC CTT CTA ACA
 S N L G C E E R A F D E N G R D L A V C
 691/231
 721/241
 TCG CCA CCA CCA CTT CCA CAT CCA CTC AAC CCA TTT TTA CAG CTC AGA CTC ACA CAG ACA
 F F C P C E D C L N F F L E V R V T D T
 751/251
 781/261
 TCG AAA CCG TCG CCG ACA CAT TTT CCG CTT CAT TCG CAG CAG CAG TTA ACC GAA TTT CCA
 F K R S R R D F C L D C D E H S T E S R
 811/271
 841/281
 TCG TCG CCG TAC CCG CTC ACA CTC CAT TTT CAA CTT TTT CCA TCG TCG CAG TCG AAT ATA CCA
 C C R Y P L T V D F E A F G W D W I I A
 871/291
 901/301
 CCG AAA ACA TAC AAA CCG AAT TAC TCG TCG CCA CAA TTT CAA TTC GTA TTT CTA CAG AAA
 P K R Y K A N Y C S C E C E F V F L G K
 931/311
 961/321
 CAG CCG CAG AAT CAG CTC CTA CAG CAA CCA AAT CCA ACA CCG TTA CCA CCG CTT TCG TCG
 Y P H T H L V M C A N P R C S A G P C C
 991/331
 1021/341
 ACA CCG ACC AAC ATG TCG CTT ATA AAC ATG CTC TAT TTC AAT CCA AAA CCA CAA ATA ATA
 T P T R M S P C N H L Y F N C K E G C C
 1051/351
 1081/361
 TAT CCA AAC ATA CCA CCG ATG CTC CTA CAT CTT TCG CCG TCG TTA TTA
 Y C K C P A M V V D R C C C S C

Turkey GDF-6

31/11
 ATG CAA AAA CTG CAA ATC TAT GTT TAT ATT TAC CTG TTT ATG CTG ATT GTT GCT CCC
 M Q K L Q I Y Y I Y L F M L I V A Q P
 61/21 91/31
 GTG GAT CTG AAT GAG AAC AGC GAG CAA AAG GAA AAT GTG GAA AAA GAG GCG CTG TGT AAT
 V D L N E N S E Q K E N V E K E G L C N
 121/41 151/51
 GCA TGT ATG TGG ACA CAA AAC ACT AAA TCT TCA ACA CTA GAA GCG ATA AAA ATT CAA ATC
 A C M W R Q N T K S S R L E A I K I Q I
 181/61 211/71
 CTC AGT AAA CTT GCG CTG GAA ACA GCT CCT AAC ATT AGC AAA GAT GCT ATA ACA CAA CTT
 L S K L R L E T A P N I S K D A I R Q L
 241/81 271/91
 TTG CCG AAA GGT GGT CCA CTC CGG GAA CTG ATT GAT CAG TAC GAT CTC CAG ACA GAT GAC
 L P K A F P L R E L I D Q Y D V Q R D D
 301/101 331/111
 AGC ACT GAT GCG TGG TGG GAA GAT GAT GAT TAT CAC GGT ACG ACG GAA ACG ATC ATT ACC
 S S D G S L E D D D Y H A T T E T I I T
 361/121 391/131
 ATG CCT ACA GAG TGT GAT CTT CTA ATG CAA GTG GAA GGA AAA GCG AAA TGG TGG TTC TTT
 M P T E S D L L M Q V E G K P K C C F F
 421/141 451/151
 AAA TTT AGC TGT AAA ATA CAA TAC AAT AAA GTA GTA AAG GCG CAA CTG TGG ATA TAT CTG
 K F S S R I Q Y N K V V K A Q L W I Y L
 481/161 511/171
 AGA CCG CTC AAG ACT CTT ACA ACA GTG TTT GTG CAA ATC CTC AGA CTC ATC AAA CCG ATG
 R P V K T P T T V F V Q I L R L I K P M
 541/181 571/191
 AAA GAC GGT ACA AGG TAT ACT GGA ATC CGA TCT CTG AAA CTT GAC ATG AAC CGA GCG ACT
 K D G T R Y T G I R S L K L D M N P G T
 601/201 631/211
 GGT ATT TGG CAG AGC ATT GAT GTG AAG ACA GTG TTG CAA AAT TGG CTC AAA CAA CTT GAA
 G I W Q S I D V R T V L Q N W L K C P E
 661/221 691/231
 TGG AAC TTA GCG ATT GAA ATC AAA GGT TTA GAT GAG AAT GGT CAT GAT CTT GGT GTA AGC
 S N L G I E I K A L D E N G H D L A V T
 721/241 751/251
 TTC CCA GGA CCA GGA GAA GAT GCG CTC AAT CCG TTT TTA GAA GTC AAG GTA ACA GAC ACA
 F P G F G E D G L N P F L E V K V T D T
 781/261 811/271
 CCA AAA AGA TGG AGG AGA GAT TTT GGA CTC GAC TGT GAT GAG CAG TCA ACA GAA TGT CGA
 F K R S E R D F G L D C D E H S T E S R
 841/281 871/291
 TGG TGT GGT TAC GTT CTA ACT GTG GAT TTT GAA GGT TTT GGA TGG GAC TGG ATT ATT GCA
 C C R Y F L T V D F E A F G W D W I I A
 901/301 931/311
 GGT AAA AGA TAT AAG GCG AAT TAC TGG TCT GGA GAG TGT GAA TTT GTA TTT TTA CAA AAA
 P K R Y K A N Y C S G E C E F V F L Q K
 961/321 991/331
 TAC CTT CAC ACT CAT CTT GTG CAC CAA GCA AAC CCG ACA GGT TCA GCA GCG CCG TGG TGT
 Y F H T H L V H Q A N P R G S A G P C C
 1021/341 1051/351
 ACT CCG ACA AAG ATG TGT CCA ATC AAT ATG CTA TAT TTT AAT GCG AAA GAA CAA ATA ATA
 T F T K M S P I N M L Y F N G K E Q I I
 1081/361 1111/371
 TAT GCG AAA ATT CCA GCG ATG GTA GTA CAT CCG TGT GCG TGG TCA TGA
 Y G K I P A M V V D R C G C S

Porcine GDF-8

FIGURE 14F

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1/1
ATG CAA AAA CTG CAA ATC TTT GTT TAT ATT TAC CTA TTT ATG CTG CTT GTT GGT GGC CCA
M Q K L Q I F V Y I Y L F M L L V A G P
61/21
CTG GAT CTG AAT GAG AAC AGC GAG CAG AAG GAA AAT GTG GAA AAA AAG GGC CTG TGT AAT
V D L N E N S E Q K E N V E K K G L C N
121/41
GCA TGC TTT TGC AGA CAA AAC AAT AAA TCC TCA AGA CTA CAA GGC ATA AAA ATC CAA ATC
A C L W R Q N N K S S R L E A I K I Q I
151/51
CTC AAT AAG CTT CGC CTG GAA ACA GCT CCG AAC ATC AGC AAA GAT GGT ATA ACA CAA CTT
L S K L R L E T A P N I S K D A I R Q L
241/81
TTG CGC AAG GGT CCG GCA CTG CGG GAA CTG ATT GAT CAG TAC GAT GTG CAG ACA GAT GAC
L P K A P P L R E L I D C Y D V Q R D D
301/101
AGC AGC GAG GGC TGC TTT GAA GAC GAT GAC TAC CAC GTT ACC AGC GAA ACC GTG ATT ACC
S S D G S L E D D D Y H V T T E T V I T
361/121
ATG CGC ACC GAG TGT GAT CTT CTA GCA GAA GTG CAA GAA AAA CGC AAA TGT TGC TTC TTT
M P T E S D L L A E V Q E K P K C C F F
421/141
AAA TTT ACC TGT AAG ATA CAA CAC AAT AAA GTA GTA AAG GCC CAA CTG TGG ATA TAT CTG
K F S S K I Q H N K V V K A Q L W I Y L
481/161
ACA CCG GTG AAG ACT CCG ACA ACA GTG TTT GTG CAA ATC CTG ACA CTC ATC AAA CGC ATG
R P V K T P T T V F V Q I L R L I K P M
541/181
AAA GAC CCG ACA AGG TAT ACT GGA ATC CGA TGT CTG AAA CTT GAC ATG AAC GCA GGC ACT
K D G T R Y T G I R S L K L D M N P G T
601/201
GGT ATT TGG CAG AGC ATT GAT GTG AAG ACA GTG TTT CAA AAC TGG CTC AAA CAA CCG GAA
G I W Q S I D V K T V L C N W L K Q P E
661/221
TTG AAC TTA GGC ATT GAA ATC AAA GGT TTA GAT GAG AAT GGT CAT GAT CTT GGT GTA ACC
S N L G I E I K A L D E N G H D L A V T
721/241
TTG CCA GAA CCA GCA GAA GAA GCA CTG AAT CCG TTT TTA GAA CTC AAG GTA ACA GAC ACA
F P E P G E E G L N P F L E V K V T D T
781/261
CCA AAA AGA TGT AGG AGA GAT TTT CGG CTT GAT TGT GAT GAG CAG TGC ACA GAA TGT CGA
P K R S R R D F G L E C C E H S T E S R
841/281
TGC TOT CCG TAC CCG CTA ACT GTG GAT TTT GAA GGT TTT GGA TGG GAT TGG ATT ATT GCA
C C R Y P L T V D F E A F G W D W I I A
901/301
GGT AAA AGA TAT AAG GGC AAT TAC TGC TGT GGA GAA TGT GAA TTT TTA TTT TTT CAA AAG
P H R Y K A N Y C S G E C E F L F L Q K
961/321
TAT CCG CAT ACC CAT CTT CTG CAC CAA GCA AAC CGC AAA GGT TCA GCC GGC CCG TGC TGT
Y P R T H L V H C A N P K G S A G P C C
1021/341
ATT CCG ACA AAG ATC TGT GCA ATT AAT ATG CTA TAT TTT AAT GGC AAA GAA CAA ATA ATA
T P T K M S P I N M L Y F N G K E Q I I
1081/361
TAT CGG AAG ATT CTA GGC ATC GTA GTA GAT CGC TGT GGC TGC TCA TCA
Y G K I P G M V V D R C C C S

```

Ovine GDF-8

FIGURE 14g

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مفتی محمد رفیع

[illegible][illegible][illegible][illegible]

L	R	P	V	K	T	F	T	T	V	F	V	Q	I	L	R	L	I	K	F	M	K	D	G	T	R	Y	T	G	I	R	S	L	K	L	D	N	S	P	G
L	B	A	G	K	T	F	T	T	V	F	V	Q	I	L	R	L	I	K	F	M	K	D	G	T	R	Y	T	G	I	R	S	L	K	L	D	N	S	P	G
L	R	P	V	E	T	F	T	T	V	F	V	Q	I	L	R	L	I	K	F	M	K	D	G	T	R	Y	T	G	I	R	S	L	K	L	D	N	H	P	G
L	R	P	V	E	T	F	T	T	V	F	V	Q	I	L	R	L	I	K	F	M	K	D	G	T	R	Y	T	G	I	R	S	L	K	L	D	N	H	P	G
L	R	P	V	K	T	F	T	T	V	F	V	Q	I	L	R	L	I	K	F	M	K	D	G	T	R	Y	T	G	I	R	S	L	K	L	D	N	H	P	G
L	R	P	V	K	T	F	T	T	V	F	V	Q	I	L	R	L	I	K	F	M	K	D	G	T	R	Y	T	G	I	R	S	L	K	L	D	N	H	P	G
L	R	P	V	K	T	F	T	T	V	F	V	Q	I	L	R	L	I	K	F	M	K	D	G	T	R	Y	T	G	I	R	S	L	K	L	D	N	H	P	G
L	B	.	V	.	.	F	T	T	V	F	V	Q	I	L	R	L	I	K	F	M	K	D	G	T	R	Y	T	G	I	R	S	L	K	L	D	N	H	P	G
L	B	.	V	.	.	F	T	T	V	F	V	Q	I	L	R	L	I	K	F	M	K	D	G	T	R	Y	T	G	I	R	S	L	K	L	D	N	H	P	G

G	I	W	Q	S	I	D	V	K	T	V	L	Q	H	W	L	R	Q	P	E	S	H	L	G	I	E	I	K	A	L	D	E	N	G	H	O	L	A	V
G	I	W	Q	S	I	D	V	K	T	V	L	Q	H	W	L	R	Q	P	E	S	H	L	G	I	E	I	K	A	L	D	E	N	G	H	O	L	A	V
G	I	W	Q	S	I	D	V	K	T	V	L	Q	H	W	L	R	Q	P	E	S	H	L	G	I	E	I	K	A	L	D	E	N	G	H	O	L	A	V
G	I	W	Q	S	I	D	V	K	T	V	L	Q	H	W	L	R	Q	P	E	S	H	L	G	I	E	I	K	A	L	D	E	N	G	H	O	L	A	V
G	I	W	Q	S	I	D	V	K	T	V	L	Q	H	W	L	R	Q	P	E	S	H	L	G	I	E	I	K	A	L	D	E	N	G	H	O	L	A	V
G	I	W	Q	S	I	D	V	K	T	V	L	Q	H	W	L	R	Q	P	E	S	H	L	G	I	E	I	K	A	L	D	E	N	G	H	O	L	A	V
G	I	W	Q	S	I	D	V	K	T	V	L	Q	H	W	L	R	Q	P	E	S	H	L	G	I	E	I	K	A	L	D	E	N	G	H	O	L	A	V
G	I	W	Q	S	I	D	V	K	T	V	L	Q	H	W	L	R	Q	P	E	S	H	L	G	I	E	I	K	A	L	D	E	N	G	H	O	L	A	V

7-00000000

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TFFGPGGEDGLHPFFLEVKVTDTFKRSRRDDEGLDCDEHSTES	human
TFFGPGGEDGLHPFFLEVKVTDTFKRSRRDDEGLDCDEHSTES	rat
TFFGPGGEDGLHPFFLEVKVTDTFKRSRRDDEGLDCDEHSTES	hamster
TFFGPGGEDGLHPFFLEVKVTDTFKRSRRDDEGLDCDEHSTES	baboon
TFFGPGGEDGLHPFFLEVKVTDTFKRSRRDDEGLDCDEHSTES	porcine
TFFGPGGEDGLHPFFLEVKVTDTFKRSRRDDEGLDCDEHSTES	ovine
TFFGPGGEDGLHPFFLEVKVTDTFKRSRRDDEGLDCDEHSTES	bovine
TFFGPGGEDGLHPFFLEVKVTDTFKRSRRDDEGLDCDEHSTES	chicken
TFFGPGGEDGLHPFFLEVKVTDTFKRSRRDDEGLDCDEHSTES	duck
RCCRYPLTVDFEAFGWQDWIIAPKRYKANYCSGECCEPVFLQ	human
RCCRYPLTVDFEAFGWQDWIIAPKRYKANYCSGECCEPVFLQ	rat
RCCRYPLTVDFEAFGWQDWIIAPKRYKANYCSGECCEPVFLQ	hamster
RCCRYPLTVDFEAFGWQDWIIAPKRYKANYCSGECCEPVFLQ	baboon
RCCRYPLTVDFEAFGWQDWIIAPKRYKANYCSGECCEPVFLQ	porcine
RCCRYPLTVDFEAFGWQDWIIAPKRYKANYCSGECCEPVFLQ	ovine
RCCRYPLTVDFEAFGWQDWIIAPKRYKANYCSGECCEPVFLQ	bovine
RCCRYPLTVDFEAFGWQDWIIAPKRYKANYCSGECCEPVFLQ	chicken
RCCRYPLTVDFEAFGWQDWIIAPKRYKANYCSGECCEPVFLQ	duck
KYFHTHLVHQAMPFRGSAGFCCTPTKHSPIIMHLYFNGKEQI	human
KYFHTHLVHQAMPFRGSAGFCCTPTKHSPIIMHLYFNGKEQI	rat
KYFHTHLVHQAMPFRGSAGFCCTPTKHSPIIMHLYFNGKEQI	hamster
KYFHTHLVHQAMPFRGSAGFCCTPTKHSPIIMHLYFNGKEQI	baboon
KYFHTHLVHQAMPFRGSAGFCCTPTKHSPIIMHLYFNGKEQI	porcine
KYFHTHLVHQAMPFRGSAGFCCTPTKHSPIIMHLYFNGKEQI	ovine
KYFHTHLVHQAMPFRGSAGFCCTPTKHSPIIMHLYFNGKEQI	bovine
KYFHTHLVHQAMPFRGSAGFCCTPTKHSPIIMHLYFNGKEQI	chicken
KYFHTHLVHQAMPFRGSAGFCCTPTKHSPIIMHLYFNGKEQI	duck
IYGKIPAMVVDRCGCS	human
IYGKIPAMVVDRCGCS	rat
IYGKIPAMVVDRCGCS	hamster
IYGKIPAMVVDRCGCS	baboon
IYGKIPAMVVDRCGCS	porcine
IYGKIPAMVVDRCGCS	ovine
IYGKIPAMVVDRCGCS	bovine
IYGKIPAMVVDRCGCS	chicken
IYGKIPAMVVDRCGCS	duck

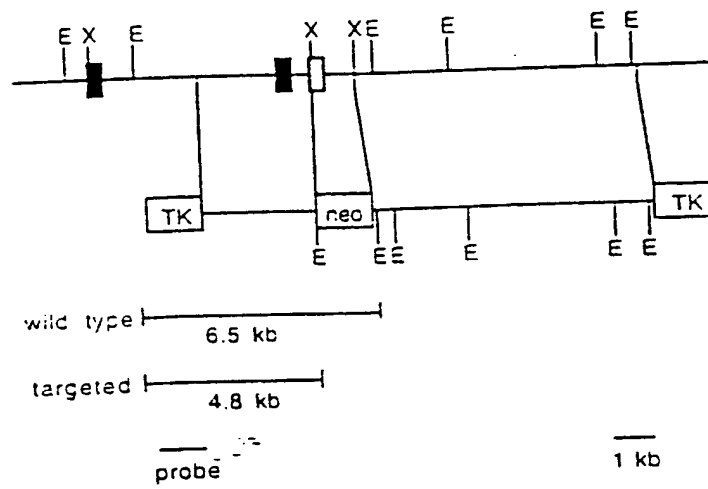
Decomposition: Decomposition #1: Shade (with solid black) residues that match the consensus exactly.

FIGURE 15b

FIGURE 16

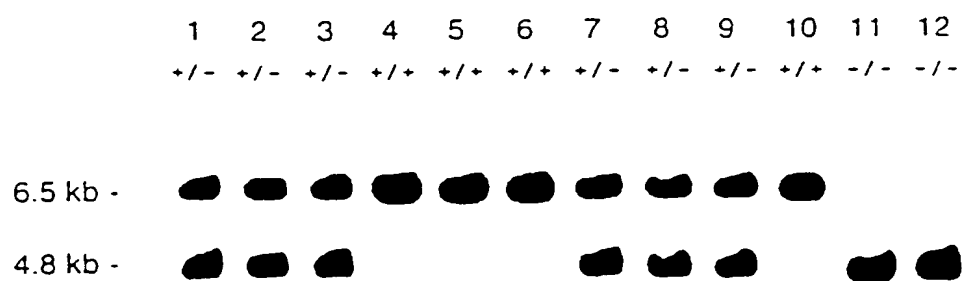
		10	20	30	40	
hGDF-11		MVLAAPLLLLGFLLLAL	ELRPRGEAAEGPAAAA	AAAAAA	AA	GVGG
hGDF-11		MVLAAPLLLLGFLLLAL	ELRPRGEAAEGPAAAA	AAAAAA	AA	GVGG
hMSTN		MAGALQMYVYLYLFML	-	-	-	IAA9VJL
hMSTN		MAGALQLYVYLYLFML	-	-	-	IVA2VJL
	50	60	70	80	90	
hGDF-11	ERSSSRPAESVAP	PEPDGCPVCVWROHS	RELRLHSIKS	QILSKLR	LRK	
hGDF-11	ERSSSRPAESVAP	PEPDGCPVCVWROHS	RELRLHSIKS	QILSKLR	LRK	
hMSTN	NEGSESESENV	EKEG-LCNACAWRQ	NTYSR	EA	IK	QILSKLR
hMSTN	NENSSESESENV	EKEG-LCNACAWRQ	NTYSR	EA	IK	QILSKLR
	100	110	120	130		
hGDF-11	EAPNISREVVVK	QLLPKAPPLQOIL	DLHDFQGDALQ	PEDFLR	EDDY	
hGDF-11	EAPNISREVVVK	QLLPKAPPLQOIL	DLHDFQGDALQ	PEDFLR	EDDY	
hMSTN	TAPNISREVVVK	QLLPKAPPLQOIL	DLHDFQGDALQ	PEDFLR	EDDY	
hMSTN	TAPNISREVVVK	QLLPKAPPLQOIL	DLHDFQGDALQ	PEDFLR	EDDY	
	140	150	160	170	180	
hGDF-11	HATTETTVISMA	QETDPAVQTDGSE	ELCCHFHES	SPKVMF	WKULKAQL	
hGDF-11	HATTETTVISMA	QETDPAVQTDGSE	ELCCHFHES	SPKVMF	WKULKAQL	
hMSTN	HATTETTVISMA	QETDPAVQTDGSE	ELCCHFHES	SPKVMF	WKULKAQL	
hMSTN	HATTETTVISMA	QETDPAVQTDGSE	ELCCHFHES	SPKVMF	WKULKAQL	
	190	200	210	220		
hGDF-11	WVYLRLPVFR	PATVYLOILRLK	PLTGEGTAGGG	GGGGRRH	IRIRSLK	
hGDF-11	WVYLRLPVFR	PATVYLOILRLK	PLTGEGTAGGG	GGGGRRH	IRIRSLK	
hMSTN	WVYLRLPVFR	PATVYLOILRLK	PLTGEGTAGGG	GGGGRRH	IRIRSLK	
hMSTN	WVYLRLPVFR	PATVYLOILRLK	PLTGEGTAGGG	GGGGRRH	IRIRSLK	
	230	240	250	260	270	
hGDF-11	IELHSRSRSGH	WQSIDFKQVLH	SWFRQEQSNW	GIEINAFD	PSGTDLA	
hGDF-11	IELHSRSRSGH	WQSIDFKQVLH	SWFRQEQSNW	GIEINAFD	PSGTDLA	
hMSTN	LOMSPGCTGI	WQSIDFKQVLH	SWFRQEQSNW	GIEINAFD	PSGTDLA	
hMSTN	LOMSPGCTGI	WQSIDFKQVLH	SWFRQEQSNW	GIEINAFD	PSGTDLA	
	280	290	300	310		
hGDF-11	VTSLGPGAEGL	HPFEMELRVLE	NTKRSRRNL	GLDCDEHS	SESRCR	
hGDF-11	VTSLGPGAEGL	HPFEMELRVLE	NTKRSRRNL	GLDCDEHS	SESRCR	
hMSTN	VTSLGPGAEGL	HPFEMELRVLE	NTKRSRRNL	GLDCDEHS	SESRCR	
hMSTN	VTSLGPGAEGL	HPFEMELRVLE	NTKRSRRNL	GLDCDEHS	SESRCR	
	320	330	340	350	360	
hGDF-11	YPLTVDPEAF	GWWDWIIAPKRY	KANYCSG	QCEYMF	MQKYPHT	HLVQ
hGDF-11	YPLTVDPEAF	GWWDWIIAPKRY	KANYCSG	QCEYMF	MQKYPHT	HLVQ
hMSTN	YPLTVDPEAF	GWWDWIIAPKRY	KANYCSG	QCEYMF	MQKYPHT	HLVQ
hMSTN	YPLTVDPEAF	GWWDWIIAPKRY	KANYCSG	QCEYMF	MQKYPHT	HLVQ
	370	380	390	400		
hGDF-11	QANPRGSAG	PCCTPTTKMS	SPINMLYFN	DKQOIIY	GKI	PGMVVDR
hGDF-11	QANPRGSAG	PCCTPTTKMS	SPINMLYFN	DKQOIIY	GKI	PGMVVDR
hMSTN	QANPRGSAG	PCCTPTTKMS	SPINMLYFN	DKQOIIY	GKI	PGMVVDR
hMSTN	QANPRGSAG	PCCTPTTKMS	SPINMLYFN	DKQOIIY	GKI	PGMVVDR
hGDF-11	CS					
hGDF-11	CS					
hMSTN	CS					
hMSTN	CS					

FIGURE 17a



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FIGURE 17b



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FIGURE 18

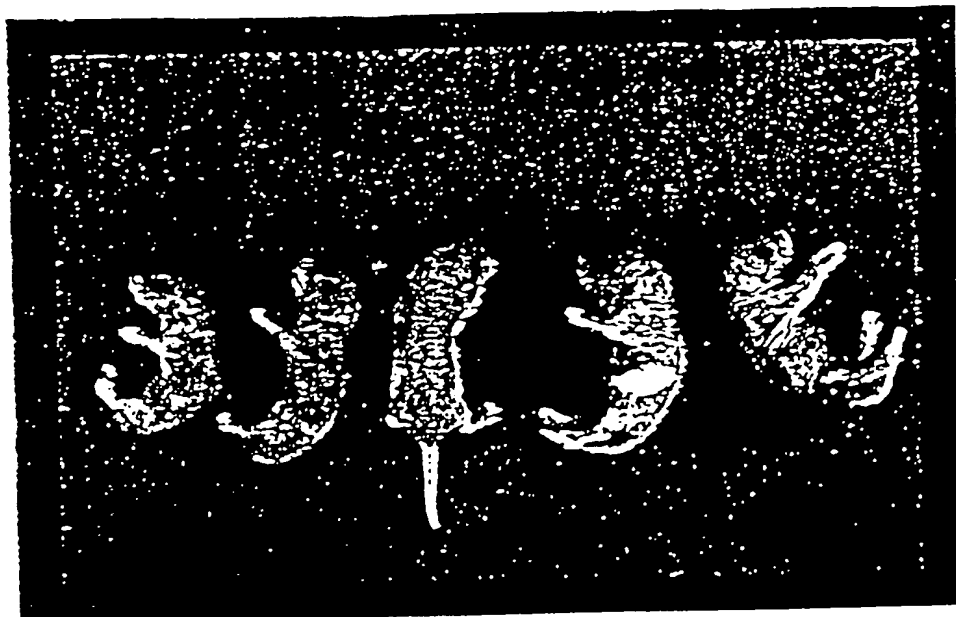
# of normal size kidneys	2	1	1	0	0	0
# of small kidneys	0	1	0	2	1	0

+ / +	47	0	0	0	0	0
+ / -	22	0	5	0	0	0
- / -	2	2	9	3	3	28

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FIGURE 19a

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FIGURE 19b



FIGURE 19c

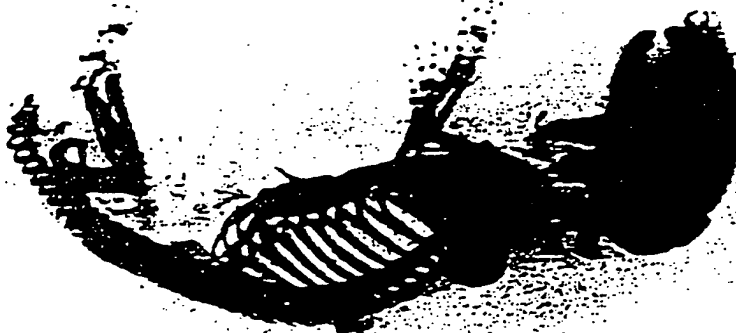
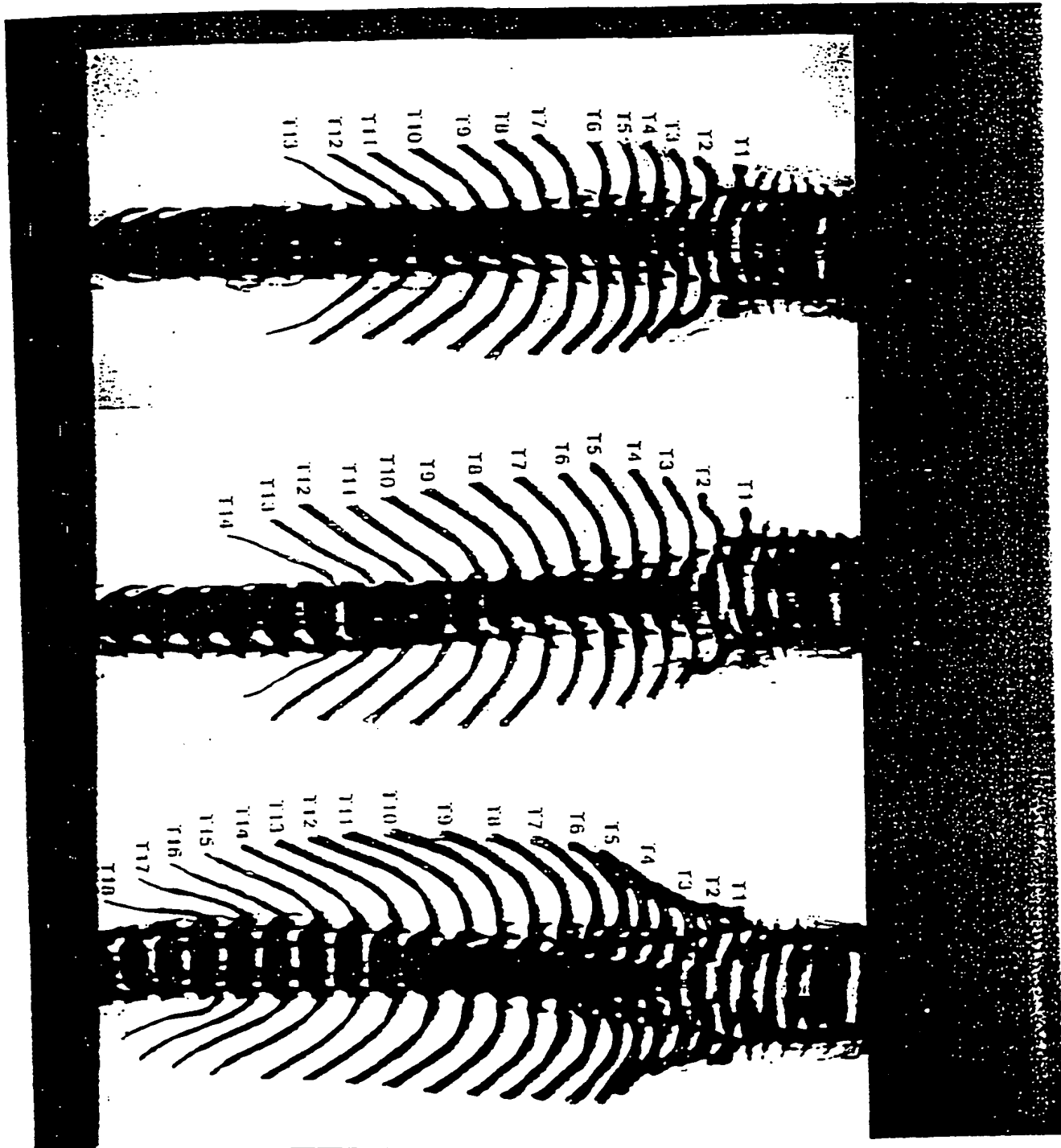


FIGURE 19d

FIG. 19e-8



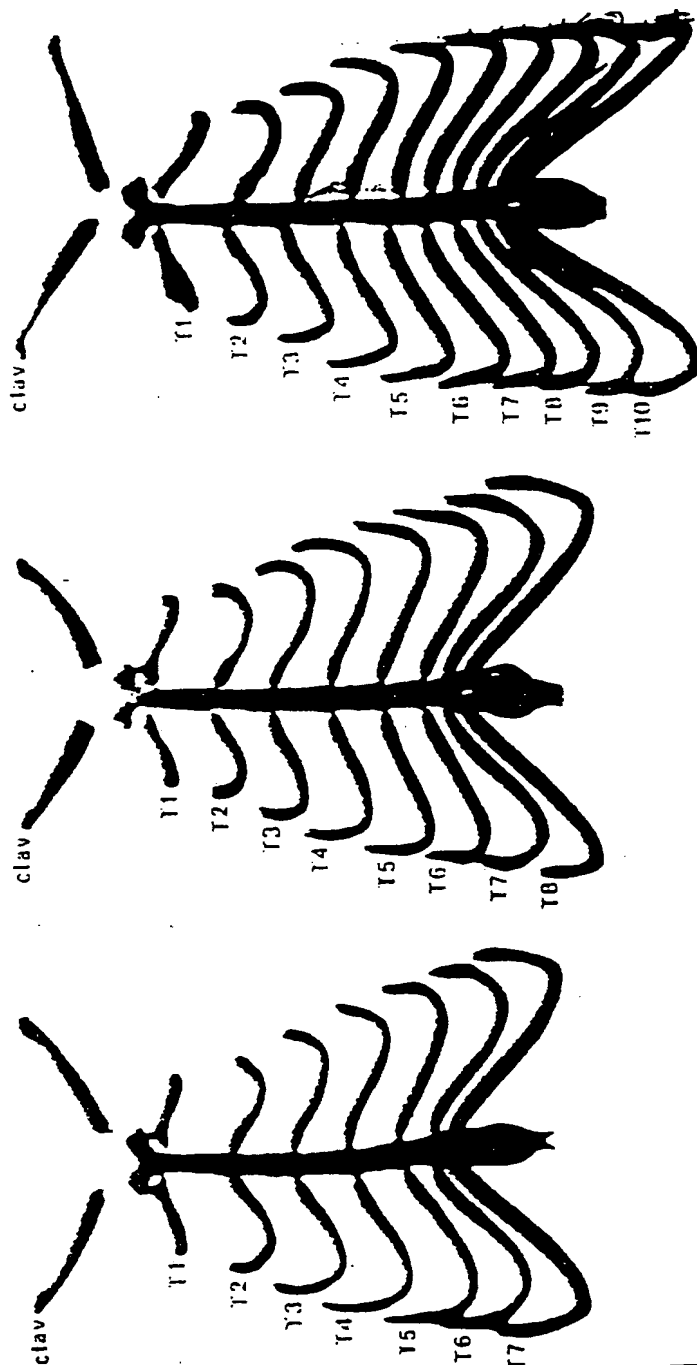


FIG. 19h-j

FIGURE 20

Table 1. Anterior transformations in wild-type, heterozygous and homozygous GDF-11 mice

	Wild-type		Heterozygous		Homozygous	
	Hybrid	129/SvJ	Hybrid	129/SvJ	Hybrid	129/SvJ
Presacral vertebrae^a						
25	4	1	-	-	-	-
26	13	6	1	-	-	-
27	-	-	59	6	-	-
33	-	-	-	-	16	2
34	-	-	-	-	5	-
Vertebral pattern^b						
C7 T13 L5	4	1	-	-	-	-
C7 T13 L6	18	5	-	-	-	-
C7 T13 L ^c	-	1	-	-	-	-
C7 T14 L5	-	-	1	-	-	-
C7 T14 L6	-	-	53	6	-	-
C7 T17 L9	-	-	-	-	-	-
C7 T18 L3	-	-	-	-	1	1
C7 T18 L9	-	-	-	-	17	1
C7 T18 L7	-	-	-	-	5	-
Anterior tuberculus on						
No vertebrae	-	1	-	-	-	-
C6	22	7	59	5	21	1
C6 and C7 ^d	-	-	-	1	2	2
Attached/unattached ribs^e						
7/6	22	8	-	-	-	-
8/6	-	-	59	6	-	-
10/7	-	-	-	-	-	1
10/8 ^f	-	-	-	-	13	2
11/6	-	-	-	-	1	-
11/7	-	-	-	-	4	-
10 + 11/8 + 7 ^g	-	-	-	-	5	-
Longest spinous process on						
T2	22	5	41	-	2	-
T3	-	-	6	6	16	-
T2 + T3 equal	-	1	8	-	1	-
T3 + T4 equal	-	-	-	-	-	2
Transitional spinous process on						
T10	22	8	3	-	-	-
T11	-	-	56	5	-	-
T12	-	-	-	-	1	-
T13	-	-	-	-	22	3
Transitional articular process on^h						
T10	22	8	1	-	-	-
T11	-	-	58	6	-	-
T13	-	-	-	-	23	3

^aVertebrae that were lumbar on one side and sacral on the other were scored as sacral. These vertebrae were seen in 2 wild-type, 3 heterozygous and 4 homozygous mutants in the hybrid background.

^bOne hybrid heterozygous, 9 hybrid homozygous and 2 129/SvJ homozygous mutants had rudimentary ribs on the most caudal thoracic segment.

^cThe number of lumbar vertebrae could not be counted due to extensive fusion of lumbar segments.

^dThese animals had a unilateral transformation of the anterior tuberculus. One 129/SvJ homozygous mutant retained one tuberculus on C6 but had bilateral tuberculus on C7.

^eOne 129/SvJ homozygous mutant had the first rib attached to the second rather than the sternum on one side only. Ten ribs were attached to the sternum on the other side.

^fRibs were asymmetrically attached.

^gOne wild-type 129/SvJ had one transitional articular process on T10 and one on T11 (scored as T10). One hybrid heterozygous mutant mice had one process on T11 and one on T12 (scored as T11).

-1-

SEQUENCE LISTING

(1) GENERAL INFORMATION

- (i) APPLICANT: Se-Jin Lee et al.,
- (ii) TITLE OF THE INVENTION: GROWTH DIFFERENTIATION FACTOR-8
- (iii) NUMBER OF SEQUENCES: 27
- (iv) CORRESPONDENCE ADDRESS:
 - (A) ADDRESSEE: Fish & Richardson P.C.
 - (B) STREET: 4225 Executive Square, Suite 1400
 - (C) CITY: La Jolla
 - (D) STATE: CA
 - (E) COUNTRY: US
 - (F) ZIP: 92037
- (v) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: Diskette
 - (B) COMPUTER: IBM Compatible
 - (C) OPERATING SYSTEM: Windows95
 - (D) SOFTWARE: FastSEQ for Windows Version 2.0
- (vi) CURRENT APPLICATION DATA:
 - (A) APPLICATION NUMBER:
 - (B) FILING DATE: 05-February-1999
 - (C) CLASSIFICATION:
- (vii) PRIOR APPLICATION DATA:
 - (A) APPLICATION NUMBER: 09/019,070
 - (B) FILING DATE: 05-February 1999
- (viii) PRIOR APPLICATION DATA:
 - (A) APPLICATION NUMBER: 09/124,180
 - (B) FILING DATE: 28-July-1998
- (viii) ATTORNEY/AGENT INFORMATION:
 - (A) NAME: Lisa A. Haile, Ph.D.
 - (B) REGISTRATION NUMBER: 38,347
 - (C) REFERENCE/DOCKET NUMBER: 07265/154WO1
- (ix) TELECOMMUNICATION INFORMATION:
 - (A) TELEPHONE: 619/678-5070
 - (B) TELEFAX: 619/678-5099

(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 35 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: Genomic DNA
- (vii) IMMEDIATE SOURCE:
 - (B) CLONE: SJL141
- (ix) FEATURE:
 - (A) NAME/KEY: Modified Base
 - (B) LOCATION: 1...35
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

-2-

CCGGAATTCG GNTGGVANRA YTGGRTNRTN NKCNC

35

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 33 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Genomic DNA

(vii) IMMEDIATE SOURCE:

- (B) CLONE: SJL147

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1...33

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

CCGGAATTCR CANSCRCARC TNTCNACNRY CAT

33

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 32 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(vii) IMMEDIATE SOURCE:

- (B) CLONE: ACM13

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1...32
- (D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

CGCGGATCCA GAAGTCAAGG TGACAGACAC AC

32

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 33 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Genomic DNA

(vii) IMMEDIATE SOURCE:

- (B) CLONE: ACM14

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1...33
- (D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

-3-

CGCGGATCCT CCTCATGAGC ACCCACAGCG GTC

33

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 550 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(vii) IMMEDIATE SOURCE:

- (B) CLONE: mouse GDF-8

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 59...436
- (D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```

TTAAGGTAGG AAGGATTTCA GGCTCTATTT ACATAATTGT TCTTTCCTTT TCACACAG      58
AAT CCC TTT TTA GAA GTC AAG GTG ACA GAC ACA CCC AAG AGG TCC CGG      106
Asn Pro Phe Leu Glu Val Lys Val Thr Asp Thr Pro Lys Arg Ser Arg
  1             5             10             15
AGA GAC TTT GGG CTT GAC TGC GAT GAG CAC TCC ACG GAA TCC CGG TGC      154
Arg Asp Phe Gly Leu Asp Cys Asp Glu His Ser Thr Glu Ser Arg Cys
             20             25             30
TGC CGC TAC CCC CTC ACG GTC GAT TTT GAA GCC TTT GGA TGG GAC TGG      202
Cys Arg Tyr Pro Leu Thr Val Asp Phe Glu Ala Phe Gly Trp Asp Trp
             35             40             45
ATT ATC GCA CCC AAA AGA TAT AAG GCC AAT TAC TGC TCA GGA GAG TGT      250
Ile Ile Ala Pro Lys Arg Tyr Lys Ala Asn Tyr Cys Ser Gly Glu Cys
             50             55             60
GAA TTT GTG TTT TTA CAA AAA TAT CCG CAT ACT CAT CTT GTG CAC CAA      298
Glu Phe Val Phe Leu Gln Lys Tyr Pro His Thr His Leu Val His Gln
             65             70             75
GCA AAC CCC AGA GGC TCA GCA GGC CCT TGC TGC ACT CCG ACA AAA ATG      346
Ala Asn Pro Arg Gly Ser Ala Gly Pro Cys Cys Thr Pro Thr Lys Met
             85             90             95
TCT CCC ATT AAT ATG CTA TAT TTT AAT GGC AAA GAA CAA ATA ATA TAT      394
Ser Pro Ile Asn Met Leu Tyr Phe Asn Gly Lys Glu Gln Ile Ile Tyr
             100            105            110
GGG AAA ATT CCA GCC ATG GTA GTA GAC CGC TGT GGG TGC TCA TGAGCTTTGC      446
Gly Lys Ile Pro Ala Met Val Val Asp Arg Cys Gly Cys Ser
             115            120            125

ATTAGGTTAG AAACTTCCCA AGTCATGGAA GGTCTTCCCC TCAATTTCGA AACTGTGAAT      506
TCCTGCAGCC CGGGGGATCC ACTAGTTCTA GAGCGGCCGC CACC                        550

```

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 126 amino acids
- (B) TYPE: amino acid

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(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```

Asn Pro Phe Leu Glu Val Lys Val Thr Asp Thr Pro Lys Arg Ser Arg
 1      5      10
Arg Asp Phe Gly Leu Asp Cys Asp Glu His Ser Thr Glu Ser Arg Cys
      20      25      30
Cys Arg Tyr Pro Leu Thr Val Asp Phe Glu Ala Phe Gly Trp Asp Trp
      35      40      45
Ile Ile Ala Pro Lys Arg Tyr Lys Ala Asn Tyr Cys Ser Gly Glu Cys
      50      55      60
Glu Phe Val Phe Leu Gln Lys Tyr Pro His Thr His Leu Val His Gln
65      70      75      80
Ala Asn Pro Arg Gly Ser Ala Gly Pro Cys Cys Thr Pro Thr Lys Met
      85      90      95
Ser Pro Ile Asn Met Leu Tyr Phe Asn Gly Lys Glu Gln Ile Ile Tyr
      100      105      110
Gly Lys Ile Pro Ala Met Val Val Asp Arg Cys Gly Cys Ser
      115      120      125

```

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 326 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(vii) IMMEDIATE SOURCE:

- (B) CLONE: human GDF-8

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 3...326
- (D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

```

CA AAA AGA TCC AGA AGG GAT TTT GGT CTT GAC TGT GAT GAG CAC TCA      47
Lys Arg Ser Arg Arg Asp Phe Gly Leu Asp Cys Asp Glu His Ser
 1      5      10      15
ACA GAA TCA CGA TGC TGT CGT TAC CCT CTA ACT GTG GAT TTT GAA GCT      95
Thr Glu Ser Arg Cys Cys Arg Tyr Pro Leu Thr Val Asp Phe Glu Ala
      20      25      30
TTT GGA TGG GAT TGG ATT ATC GCT CCT AAA AGA TAT AAG GCC AAT TAC      143
Phe Gly Trp Asp Trp Ile Ile Ala Pro Lys Arg Tyr Lys Ala Asn Tyr
      35      40      45
TGC TCT GGA GAG TGT GAA TTT GTA TTT TTA CAA AAA TAT CCT CAT ACT      191
Cys Ser Gly Glu Cys Glu Phe Val Phe Leu Gln Lys Tyr Pro His Thr
      50      55      60
CAT CTG GTA CAC CAA GCA AAC CCC AGA GGT TCA GCA GGC CCT TGC TGT      239
His Leu Val His Gln Ala Asn Pro Arg Gly Ser Ala Gly Pro Cys Cys
      65      70      75

```

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ACT CCC ACA AAG ATG TCT CCA ATT AAT ATG CTA TAT TTT AAT GGC AAA 287
 Thr Pro Thr Lys Met Ser Pro Ile Asn Met Leu Tyr Phe Asn Gly Lys
 80 85 90 95

GAA CAA ATA ATA TAT GGG AAA ATT CCA GCG ATG GTA GTA 326
 Glu Gln Ile Ile Tyr Gly Lys Ile Pro Ala Met Val Val
 100 105

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 108 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Lys Arg Ser Arg Arg Asp Phe Gly Leu Asp Cys Asp Glu His Ser Thr
 1 5 10 15
 Glu Ser Arg Cys Cys Arg Tyr Pro Leu Thr Val Asp Phe Glu Ala Phe
 20 25 30
 Gly Trp Asp Trp Ile Ile Ala Pro Lys Arg Tyr Lys Ala Asn Tyr Cys
 35 40 45
 Ser Gly Glu Cys Glu Phe Val Phe Leu Gln Lys Tyr Pro His Thr His
 50 55 60
 Leu Val His Gln Ala Asn Pro Arg Gly Ser Ala Gly Pro Cys Cys Thr
 65 70 75 80
 Pro Thr Lys Met Ser Pro Ile Asn Met Leu Tyr Phe Asn Gly Lys Glu
 85 90 95
 Gln Ile Ile Tyr Gly Lys Ile Pro Ala Met Val Val
 100 105

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 9 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vii) IMMEDIATE SOURCE:

- (B) CLONE: SJL141

(ix) FEATURE:

- (A) NAME/KEY: Peptide
 (B) LOCATION: 1...9
 (D) OTHER INFORMATION: "Xaa at position 3 = His, Gln, Asn, Lys, Asp, or Glu; Xaa at position 4 = Asp or Asn; Xaa at positions 6 and 7 is Val, Ile, or Met; Xaa at position 8 = Ala or Ser."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9

Gly Trp Xaa Xaa Trp Xaa Xaa Xaa Pro
 1 5

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

-6-

- (A) LENGTH: 8 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vii) IMMEDIATE SOURCE:
 (B) CLONE: SJL147

(ix) FEATURE:

- (A) NAME/KEY: Peptide
- (B) LOCATION: 1...8
- (D) OTHER INFORMATION: "Xaa at position 2 = Val, Ile, Met, Thr or Ala; Xaa at position 4 = Asp or Glu; Xaa at position 7 = Gly, or Ala.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Met Xaa Val Xaa Ser Cys Xaa Cys
 1 5

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2676 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Genomic DNA

(vii) IMMEDIATE SOURCE:
 (B) CLONE: Murine GDF-8

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1...2676
- (D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

GTCTCTCGGA	CGGTACATGC	ACTAATATTT	CACTTGGCAT	TACTCAAAAG	CAAAAAGAAG	60
AAATAAGAAC	AAGGGAAAAA	AAAAGATTGT	GCTGATTTT	AAA ATG ATG CAA AAA		115
				Met Met Gln Lys		
				1		
CTG CAA ATG TAT GTT TAT ATT TAC CTG TTC ATG CTG ATT GCT GCT GGC						163
Leu Gln Met Tyr Val Tyr Ile Tyr Leu Phe Met Leu Ile Ala Ala Gly						
5 10 15 20						
CCA GTG GAT CTA AAT GAG GGC AGT GAG AGA GAA GAA AAT GTG GAA AAA						211
Pro Val Asp Leu Asn Glu Gly Ser Glu Arg Glu Glu Asn Val Glu Lys						
25 30 35						
GAG GGG CTG TGT AAT GCA TGT GCG TGG AGA CAA AAC ACG AGG TAC TCC						259
Glu Gly Leu Cys Asn Ala Cys Ala Trp Arg Gln Asn Thr Arg Tyr Ser						
40 45 50						
AGA ATA GAA GCC ATA AAA ATT CAA ATC CTC AGT AAG CTG CGC CTG GAA						307
Arg Ile Glu Ala Ile Lys Ile Gln Ile Leu Ser Lys Leu Arg Leu Glu						
55 60 65						
ACA GCT CCT AAC ATC AGC AAA GAT GCT ATA AGA CAA CTT CTG CCA AGA						355
Thr Ala Pro Asn Ile Ser Lys Asp Ala Ile Arg Gln Leu Leu Pro Arg						
70 75 80						

-7-

GCG CCT CCA CTC CGG GAA CTG ATC GAT CAG TAC GAC GTC CAG AGG GAT Ala Pro Pro Leu Arg Glu Leu Ile Asp Gln Tyr Asp Val Gln Arg Asp 85 90 95 100	403
GAC AGC AGT GAT GGC TCT TTG GAA GAT GAC GAT TAT CAC GCT ACC ACG Asp Ser Ser Asp Gly Ser Leu Glu Asp Asp Asp Tyr His Ala Thr Thr 105 110 115	451
GAA ACA ATC ATT ACC ATG CCT ACA GAG TCT GAC TTT CTA ATG CAA GCG Glu Thr Ile Ile Thr Met Pro Thr Glu Ser Asp Phe Leu Met Gln Ala 120 125 130	499
GAT GGC AAG CCC AAA TGT TGC TTT TTT AAA TTT AGC TCT AAA ATA CAG Asp Gly Lys Pro Lys Cys Cys Phe Phe Lys Phe Ser Ser Lys Ile Gln 135 140 145	547
TAC AAC AAA GTA GTA AAA GCC CAA CTG TGG ATA TAT CTC AGA CCC GTC Tyr Asn Lys Val Val Lys Ala Gln Leu Trp Ile Tyr Leu Arg Pro Val 150 155 160	595
AAG ACT CCT ACA ACA GTG TTT GTG CAA ATC CTG AGA CTC ATC AAA CCC Lys Thr Pro Thr Thr Val Phe Val Gln Ile Leu Arg Leu Ile Lys Pro 165 170 175 180	643
ATG AAA GAC GGT ACA AGG TAT ACT GGA ATC CGA TCT CTG AAA CTT GAC Met Lys Asp Gly Thr Arg Tyr Thr Gly Ile Arg Ser Leu Lys Leu Asp 185 190 195	691
ATG AGC CCA GGC ACT GGT ATT TGG CAG AGT ATT GAT GTG AAG ACA GTG Met Ser Pro Gly Thr Gly Ile Trp Gln Ser Ile Asp Val Lys Thr Val 200 205 210	739
TTG CAA AAT TGG CTC AAA CAG CCT GAA TCC AAC TTA GGC ATT GAA ATC Leu Gln Asn Trp Leu Lys Gln Pro Glu Ser Asn Leu Gly Ile Glu Ile 215 220 225	787
AAA GCT TTG GAT GAG AAT GGC CAT GAT CTT GCT GTA ACC TTC CCA GGA Lys Ala Leu Asp Glu Asn Gly His Asp Leu Ala Val Thr Phe Pro Gly 230 235 240	835
CCA GGA GAA GAT GGG CTG AAT CCC TTT TTA GAA GTC AAG GTG ACA GAC Pro Gly Glu Asp Gly Leu Asn Pro Phe Leu Glu Val Lys Val Thr Asp 245 250 255 260	883
ACA CCC AAG AGG TCC CGG AGA GAC TTT GGG CTT GAC TGC GAT GAG CAC Thr Pro Lys Arg Ser Arg Arg Asp Phe Gly Leu Asp Cys Asp Glu His 265 270 275	931
TCC ACG GAA TCC CGG TGC TGC CGC TAC CCC CTC ACG GTC GAT TTT GAA Ser Thr Glu Ser Arg Cys Cys Arg Tyr Pro Leu Thr Val Asp Phe Glu 280 285 290	979
GCC TTT GGA TGG GAC TGG ATT ATC GCA CCC AAA AGA TAT AAG GCC AAT Ala Phe Gly Trp Asp Trp Ile Ile Ala Pro Lys Arg Tyr Lys Ala Asn 295 300 305	1027
TAC TGC TCA GGA GAG TGT GAA TTT GTG TTT TTA CAA AAA TAT CCG CAT Tyr Cys Ser Gly Glu Cys Glu Phe Val Phe Leu Gln Lys Tyr Pro His 310 315 320	1075
ACT CAT CTT GTG CAC CAA GCA AAC CCC AGA GGC TCA GCA GGC CCT TGC Thr His Leu Val His Gln Ala Asn Pro Arg Gly Ser Ala Gly Pro Cys 325 330 335 340	1123

- 8 -

TGC ACT CCG ACA AAA ATG TCT CCC ATT AAT ATG CTA TAT TTT AAT GGC 1171
 Cys Thr Pro Thr Lys Met Ser Pro Ile Asn Met Leu Tyr Phe Asn Gly
 345 350 355

AAA GAA CAA ATA ATA TAT GGG AAA ATT CCA GCC ATG GTA GTA GAC CGC 1219
 Lys Glu Gln Ile Ile Tyr Gly Lys Ile Pro Ala Met Val Val Asp Arg
 360 365 370

TGT GGG TGC TCA TGAGCTTTGC ATTAGGTTAG AAACCTTCCCA AGTCATGGAA GGTCT 1276
 Cys Gly Cys Ser

375

TCCCCTCAAT TTCGAAACTG TGAATTCAAG CACCACAGGC TGTAGGCCTT GAGTATGCTC 1336
 TAGTAACGTA AGCACAAAGCT ACAGTGTATG AACTAAAAGA GAGAATAGAT GCAATGGTTG 1396
 GCATTCAACC ACCAAAATAA ACCATACTAT AGGATGTTGT ATGATTTCCA GAGTTTTTGA 1456
 AATAGATGGA GATCAAATTA CATTATGTC CATATATGTA TATTACAAC ACAAATCTAGG 1516
 CAAGGAAGTG AGAGCACATC TTGTGGTCTG CTGAGTTAGG AGGGTATGAT TAAAAGGTAA 1576
 AGTCTTATTT CCTAACAGTT TCACTTAATA TTTACAGAAG AATCTATATG TAGCCTTTGT 1636
 AAAGTGTAGG ATTGTTATCA TTTAAAAACA TCATGTACAC TTATATTTGT ATTGTATACT 1696
 TGGTAAGATA AAATCCACA AAGTAGGAAT GGGGCCTCAC ATACACATTG CCATTCCTAT 1756
 TATAATTGGA CAATCCACCA CGGTGCTAAT GCAGTGCTGA ATGGCTCCTA CTGGACCTCT 1816
 CGATAGAACA CTCTACAAAG TACGAGTCTC TCTCTCCCTT CCAGGTGCAT CTCCACACAC 1876
 ACAGCACTAA GTGTTCAATG CATTTCCTTT AAGGAAAAGAA GAATCTTTTT TTCTAGAGGT 1936
 CAACCTTCAG TCAACTCTAG CACAGCGGGA GTGACTGCTG CATCTTAAAA GGCAGCCAAA 1996
 CAGTATTCAT TTTTAAATCT AAATTTCAAA ATCACTGTCT GCCTTTATCA CATGGCAATT 2056
 TTGTGGTAAA ATAATGGAAA TGAAGTGGTT TATCAATATT GTATAAAAGA CTCTGAAACA 2116
 ATTACATTTA TATAATATGT ATACAATATT GTTTTGTAAG TAAGTGTCTC CTTTTATATT 2176
 TACTTTGGTA TATTTTACCA CTAATGAAAT TTCAAATCAT TAAAGTACAA AGACATGTCA 2236
 TGTATCACAA AAAAGGTGAC TGCTTCTATT TCAGAGTGAA TTAGCAGATT CAATAGTGGT 2296
 CTTAAAACTC TGTATGTTAA GATTAGAAGG TTATATTACA ATCAATTTAT GTATTTTTTA 2356
 CATTATCAAC TTATGGTTTC ATGGTGGCTG TATCTATGAA TGTGGCTCCC AGTCAAATTT 2416
 CAATGCCCCA CCATTTTAAA AATTACAAGC ATTACTAAAC ATACCAACAT GTATCTAAAG 2476
 AAATACAAAT ATGGTATCTC AATAACAGCT ACTTTTTTAT TTTATAATTT GACAATGAAT 2536
 ACATTTCTTT TATTTACTTC AGTTTTATAA ATTGGAACCT TGTATTATCA ATGTATTGTA 2596
 TCATAGCTA AATGAAATTA TTTCTTACAT AAAAATGTGT AGAACTATA AATTAAAGTG 2656
 TTTTCACATT TTTGAAAGGC 2676

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 376 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Met Met Gln Lys Leu Gln Met Tyr Val Tyr Ile Tyr Leu Phe Met Leu
 1 5 10 15
 Ile Ala Ala Gly Pro Val Asp Leu Asn Glu Gly Ser Glu Arg Glu Glu
 20 25 30
 Asn Val Glu Lys Glu Gly Leu Cys Asn Ala Cys Ala Trp Arg Gln Asn
 35 40 45
 Thr Arg Tyr Ser Arg Ile Glu Ala Ile Lys Ile Gln Ile Leu Ser Lys
 50 55 60
 Leu Arg Leu Glu Thr Ala Pro Asn Ile Ser Lys Asp Ala Ile Arg Gln
 65 70 75 80
 Leu Leu Pro Arg Ala Pro Pro Leu Arg Glu Leu Ile Asp Gln Tyr Asp
 85 90 95
 Val Gln Arg Asp Ser Ser Asp Gly Ser Leu Glu Asp Asp Asp Tyr
 100 105 110
 His Ala Thr Thr Glu Thr Ile Ile Thr Met Pro Thr Glu Ser Asp Phe
 115 120 125

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Leu Met Gln Ala Asp Gly Lys Pro Lys Cys Cys Phe Phe Lys Phe Ser
 130 135 140
 Ser Lys Ile Gln Tyr Asn Lys Val Val Lys Ala Gln Leu Trp Ile Tyr
 145 150 155 160
 Leu Arg Pro Val Lys Thr Pro Thr Thr Val Phe Val Gln Ile Leu Arg
 165 170 175
 Leu Ile Lys Pro Met Lys Asp Gly Thr Arg Tyr Thr Gly Ile Arg Ser
 180 185 190
 Leu Lys Leu Asp Met Ser Pro Gly Thr Gly Ile Trp Gln Ser Ile Asp
 195 200 205
 Val Lys Thr Val Leu Gln Asn Trp Leu Lys Gln Pro Glu Ser Asn Leu
 210 215 220
 Gly Ile Glu Ile Lys Ala Leu Asp Glu Asn Gly His Asp Leu Ala Val
 225 230 235 240
 Thr Phe Pro Gly Pro Gly Glu Asp Gly Leu Asn Pro Phe Leu Glu Val
 245 250 255
 Lys Val Thr Asp Thr Pro Lys Arg Ser Arg Arg Asp Phe Gly Leu Asp
 260 265 270
 Cys Asp Glu His Ser Thr Glu Ser Arg Cys Cys Arg Tyr Pro Leu Thr
 275 280 285
 Val Asp Phe Glu Ala Phe Gly Trp Asp Trp Ile Ile Ala Pro Lys Arg
 290 295 300
 Tyr Lys Ala Asn Tyr Cys Ser Gly Glu Cys Glu Phe Val Phe Leu Gln
 305 310 315 320
 Lys Tyr Pro His Thr His Leu Val His Gln Ala Asn Pro Arg Gly Ser
 325 330 335
 Ala Gly Pro Cys Cys Thr Pro Thr Lys Met Ser Pro Ile Asn Met Leu
 340 345 350
 Tyr Phe Asn Gly Lys Glu Gln Ile Ile Tyr Gly Lys Ile Pro Ala Met
 355 360 365
 Val Val Asp Arg Cys Gly Cys Ser
 370 375

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2743 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Genomic DNA

(vii) IMMEDIATE SOURCE:

- (B) CLONE: Human GDF-8

(ix) FEATURE:

- (A) NAME/KEY: CDS
 (B) LOCATION: 1...2743
 (D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

AAGAAAAGTA AAAGGAAGAA ACAAGAACAA GAAAAAAGAT TATATTGATT TTAAAATC 58
 ATG CAA AAA CTG CAA CTC TGT GTT TAT ATT TAC CTG TTT ATG CTG ATT 106
 Met Gln Lys Leu Gln Leu Cys Val Tyr Ile Tyr Leu Phe Met Leu Ile 15
 1 5 10
 GTT GCT GGT CCA GTG GAT CTA AAT GAG AAC AGT GAG CAA AAA GAA AAT 154
 Val Ala Gly Pro Val Asp Leu Asn Glu Asn Ser Glu Gln Lys Glu Asn 20 25 30

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GTG	GAA	AAA	GAG	GGG	CTG	TGT	AAT	GCA	TGT	ACT	TGG	AGA	CAA	AAC	ACT	202
Val	Glu	Lys	Glu	Gly	Leu	Cys	Asn	Ala	Cys	Thr	Trp	Arg	Gln	Asn	Thr	
35					40					45						
AAA	TCT	TCA	AGA	ATA	GAA	GCC	ATT	AAG	ATA	CAA	ATC	CTC	AGT	AAA	CTT	250
Lys	Ser	Ser	Arg	Ile	Glu	Ala	Ile	Lys	Ile	Gln	Ile	Leu	Ser	Lys	Leu	
	50					55					60					
CGT	CTG	GAA	ACA	GCT	CCT	AAC	ATC	AGC	AAA	GAT	GTT	ATA	AGA	CAA	CTT	298
Arg	Leu	Glu	Thr	Ala	Pro	Asn	Ile	Ser	Lys	Asp	Val	Ile	Arg	Gln	Leu	
65					70					75					80	
TTA	CCC	AAA	GCT	CCT	CCA	CTC	CGG	GAA	CTG	ATT	GAT	CAG	TAT	GAT	GTC	346
Leu	Pro	Lys	Ala	Pro	Pro	Leu	Arg	Glu	Leu	Ile	Asp	Gln	Tyr	Asp	Val	
				85					90					95		
CAG	AGG	GAT	GAC	AGC	AGC	GAT	GGC	TCT	TTG	GAA	GAT	GAC	GAT	TAT	CAC	394
Gln	Arg	Asp	Asp	Ser	Ser	Asp	Gly	Ser	Leu	Glu	Asp	Asp	Asp	Tyr	His	
			100					105					110			
GCT	ACA	ACG	GAA	ACA	ATC	ATT	ACC	ATG	CCT	ACA	GAG	TCT	GAT	TTT	CTA	442
Ala	Thr	Thr	Glu	Thr	Ile	Ile	Thr	Met	Pro	Thr	Glu	Ser	Asp	Phe	Leu	
		115					120					125				
ATG	CAA	GTG	GAT	GGA	AAA	CCC	AAA	TGT	TGC	TTC	TTT	AAA	TTT	AGC	TCT	490
Met	Gln	Val	Asp	Gly	Lys	Pro	Lys	Cys	Cys	Phe	Phe	Lys	Phe	Ser	Ser	
	130					135					140					
AAA	ATA	CAA	TAC	AAT	AAA	GTA	GTA	AAG	GCC	CAA	CTA	TGG	ATA	TAT	TTG	538
Lys	Ile	Gln	Tyr	Asn	Lys	Val	Val	Lys	Ala	Gln	Leu	Trp	Ile	Tyr	Leu	
145					150					155					160	
AGA	CCC	GTC	GAG	ACT	CCT	ACA	ACA	GTG	TTT	GTG	CAA	ATC	CTG	AGA	CTC	586
Arg	Pro	Val	Glu	Thr	Pro	Thr	Thr	Val	Phe	Val	Gln	Ile	Leu	Arg	Leu	
				165					170					175		
ATC	AAA	CCT	ATG	AAA	GAC	GGT	ACA	AGG	TAT	ACT	GGA	ATC	CGA	TCT	CTG	634
Ile	Lys	Pro	Met	Lys	Asp	Gly	Thr	Arg	Tyr	Thr	Gly	Ile	Arg	Ser	Leu	
			180					185					190			
AAA	CTT	GAC	ATG	AAC	CCA	GGC	ACT	GGT	ATT	TGG	CAG	AGC	ATT	GAT	GTG	682
Lys	Leu	Asp	Met	Asn	Pro	Gly	Thr	Gly	Ile	Trp	Gln	Ser	Ile	Asp	Val	
		195				200						205				
AAG	ACA	GTG	TTG	CAA	AAT	TGG	CTC	AAA	CAA	CCT	GAA	TCC	AAC	TTA	GGC	730
Lys	Thr	Val	Leu	Gln	Asn	Trp	Leu	Lys	Gln	Pro	Glu	Ser	Asn	Leu	Gly	
	210					215					220					
ATT	GAA	ATA	AAA	GCT	TTA	GAT	GAG	AAT	GGT	CAT	GAT	CTT	GCT	GTA	ACC	778
Ile	Glu	Ile	Lys	Ala	Leu	Asp	Glu	Asn	Gly	His	Asp	Leu	Ala	Val	Thr	
225					230					235					240	
TTC	CCA	GGA	CCA	GGA	GAA	GAT	GGG	CTG	AAT	CCG	TTT	TTA	GAG	GTC	AAG	826
Phe	Pro	Gly	Pro	Gly	Glu	Asp	Gly	Leu	Asn	Pro	Phe	Leu	Glu	Val	Lys	
				245					250					255		
GTA	ACA	GAC	ACA	CCA	AAA	AGA	TCC	AGA	AGG	GAT	TTT	GGT	CTT	GAC	TGT	874
Val	Thr	Asp	Thr	Pro	Lys	Arg	Ser	Arg	Arg	Asp	Phe	Gly	Leu	Asp	Cys	
			260					265					270			
GAT	GAG	CAC	TCA	ACA	GAA	TCA	CGA	TGC	TGT	CGT	TAC	CCT	CTA	ACT	GTG	922
Asp	Glu	His	Ser	Thr	Glu	Ser	Arg	Cys	Cys	Arg	Tyr	Pro	Leu	Thr	Val	
		275					280					285				

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GAT TTT GAA GCT TTT GGA TGG GAT TGG ATT ATC GCT CCT AAA AGA TAT	970
Asp Phe Glu Ala Phe Gly Trp Asp Trp Ile Ile Ala Pro Lys Arg Tyr	
290 295 300	
AAG GCC AAT TAC TGC TCT GGA GAG TGT GAA TTT GTA TTT TTA CAA AAA	1018
Lys Ala Asn Tyr Cys Ser Gly Glu Cys Glu Phe Val Phe Leu Gln Lys	
305 310 315 320	
TAT CCT CAT ACT CAT CTG GTA CAC CAA GCA AAC CCC AGA GGT TCA GCA	1066
Tyr Pro His Thr His Leu Val His Gln Ala Asn Pro Arg Gly Ser Ala	
325 330 335	
GGC CCT TGC TGT ACT CCC ACA AAG ATG TCT CCA ATT AAT ATG CTA TAT	1114
Gly Pro Cys Cys Thr Pro Thr Lys Met Ser Pro Ile Asn Met Leu Tyr	
340 345 350	
TTT AAT GGC AAA GAA CAA ATA ATA TAT GGG AAA ATT CCA GCG ATG GTA	1162
Phe Asn Gly Lys Glu Gln Ile Ile Tyr Gly Lys Ile Pro Ala Met Val	
355 360 365	
GTA GAC CGC TGT GGG TGC TCA TGAGATTTAT ATTAAGCGTT CATAACTTCC TAAAA	1219
Val Asp Arg Cys Gly Cys Ser	
370 375	
ATGGAAGGTT TTCCCCTCAA CAATTTTGAA GCTGTGAAAT TAAGTACCAC AGGCTATAGG	1279
CCTAGAGTAT GCTACAGTCA CTTAAGCATA AGCTACAGTA TGTAAGCTAA AAGGGGGAAT	1339
ATATGCAATG GTTGGCATT T AACCATCCAA ACAAATCATA CAAGAAAGTT TTATGATTTT	1399
CAGAGTTTTT GAGCTAGAAG GAGATCAAAT TACATTTATG TTCCTATATA TTACAACATC	1459
GGCGAGGAAA TGAAAGCGAT TCTCCTTGAG TTCTGATGAA TTAAAGGAGT ATGCTTTAAA	1519
GTCTATTTCT TTAAAGTTTT GTTTAATATT TACAGAAAAA TCCACATACA GTATTGGTAA	1579
AATGCAGGAT TGTTATATAC CATCATTCGA ATCATCCTTA AACACTTGAA TTTATATTGT	1639
ATGGTAGTAT ACTTGGTAAG ATAAAATTCC ACAAAAATAG GGATGGTGCA GCATATGCAA	1699
TTTCCATTCC TATTATAATT GACACAGTAC ATTAACAATC CATGCCAACG GTGCTAATAC	1759
GATAGGCTGA ATGTCTGAGG CTACCAGGTT TATCACATAA AAAACATTCA GTAAAATAGT	1819
AAGTTTCTCT TTTCTTCAGG TGCATTTTCC TACACCTCCA AATGAGGAAT GGATTTTCTT	1879
TAATGTAAGA AGAATCATTT TTCTAGAGGT TGGCTTTCAA TTCTGTAGCA TACTTGGAGA	1939
AACTGCATTA TCTTAAAAGG CAGTCAAATG GTGTTTGT TTATCAAAAT GTCAAAATAA	1999
CATACTTGGA GAAGTATGTA ATTTTGTCTT TGGAAAATTA CAACACTGCC TTTGCAACAC	2059
TGCAGTTTTT ATGGTAAAAT AATAGAAATG ATCGACTCTA TCAATATTGT ATAAAAAGAC	2119
TGAAACAATG CATTTATATA ATATGTATAC AATATTGTTT TGTAATAAAG TGTCTCCTTT	2179
TTTATTTACT TTGGTATATT TTTACACTAA GGACATTTCA AATTAAGTAC TAAGGCACAA	2239
AGACATGTCA TGCATCACAG AAAAGCAACT ACTTATATTT CAGAGCAAAT TAGCAGATTA	2299
AATAGTGGTC TTAAAACTCC ATATGTTAAT GATTAGATGG TTATATTACA ATCATTTTAT	2359
ATTTTTTTTAC ATGATTAACA TTTACTTATG GATTCTATG GGCTGTATAA AGTGAATTTG	2419
AAATTTCAAT GGTTTACTGT CATTTGTGTT AAATCTCAAC GTTCCATTAT TTTAATACTT	2479
GCAAAAACAT TACTAAGTAT ACCAAAATAA TTGACTCTAT TATCTGAAAT GAAGAATAAA	2539
CTGATGCTAT CTCAACAATA ACTGTTACTT TTATTTTATA ATTTGATAAT GAATATATTT	2599
CTGCATTTAT TTTACTTCTGT TTTGTAAATT GGGATTTTGT TAATCAAATT TATTGTACTA	2659
TGACTAAATG AAATTATTTT TTACATCTAA TTTGTAGAAA CAGTATAAGT TATATTAAAG	2719
TGTTTTTACA TTTTTTTGAA AGAC	2743

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 375 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

(A) LENGTH: 34 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(B) CLONE: #83

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(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..34
- (C) OTHER:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

CGCGGATCCG TGGATCTAAA TGAGAACAGT GAGC

34

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 37 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Genomic DNA

(vii) IMMEDIATE SOURCE:

- (B) CLONE: #84

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1...37
- (C) OTHER:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

CGCGAATTCT CAGGTAATGA TTGTTTCCGT TGTAGCG

37

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Genomic DNA

(vii) IMMEDIATE SOURCE:

- (B) CLONE: #100

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1...20
- (C) OTHER:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

ACACTAAATC TTCAAGAATA

20

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1055 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Genomic DNA

(vii) IMMEDIATE SOURCE:

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(B) CLONE: Baboon GDF-8

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 1...1055

(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

									ATG	CAA	AAA					9
									Met	Gln	Lys					
									1							
CTG	CAA	CTC	TGT	GTT	TAT	ATT	TAC	CTG	TTT	ATG	CTG	ATT	GTT	GCT	GGT	54
Leu	Gln	Leu	Cys	Val	Tyr	Ile	Tyr	Leu	Phe	Met	Leu	Ile	Val	Ala	Gly	
5					10					15					20	
CCA	GTG	GAT	CTA	AAT	GAG	AAC	AGT	GAG	CAA	AAA	GAA	AAT	GTG	GAA	AAA	95
Pro	Val	Asp	Leu	Asn	Glu	Asn	Ser	Glu	Gln	Lys	Glu	Asn	Val	Glu	Lys	
				25					30					35		
GAG	GGG	CTG	TGT	AAT	GCA	TGT	ACT	TGG	AGA	CAA	AAC	ACT	AAA	TCT	TCA	140
Glu	Gly	Leu	Cys	Asn	Ala	Cys	Thr	Trp	Arg	Gln	Asn	Thr	Lys	Ser	Ser	
			40					45					50			
AGA	ATA	GAA	GCC	ATT	AAA	ATA	CAA	ATC	CTC	AGT	AAA	CTT	CGT	CTG	GAA	185
Arg	Ile	Glu	Ala	Ile	Lys	Ile	Gln	Ile	Leu	Ser	Lys	Leu	Arg	Leu	Glu	
		55					60					65				
ACA	GCT	CCT	AAC	ATC	AGC	AAA	GAT	GCT	ATA	AGA	CAA	CTT	TTA	CCC	AAA	230
Thr	Ala	Pro	Asn	Ile	Ser	Lys	Asp	Ala	Ile	Arg	Gln	Leu	Leu	Pro	Lys	
	70					75					80					
GCG	CCT	CCA	CTC	CGG	GAA	CTG	ATT	GAT	CAG	TAT	GAT	GTC	CAG	AGG	GAT	275
Ala	Pro	Pro	Leu	Arg	Glu	Leu	Ile	Asp	Gln	Tyr	Asp	Val	Gln	Arg	Asp	
	85				90					95					100	
GAC	AGC	AGC	GAT	GGC	TCT	TTG	GAA	GAT	GAC	GAT	TAT	CAC	GCT	ACA	ACG	320
Asp	Ser	Ser	Asp	Gly	Ser	Leu	Glu	Asp	Asp	Asp	Tyr	His	Ala	Thr	Thr	
				105					110					115		
GAA	ACA	ATC	ATT	ACC	ATG	CCT	ACA	GAG	TCT	GAT	TTT	TTA	ATG	CAA	GTG	365
Glu	Thr	Ile	Ile	Thr	Met	Pro	Thr	Glu	Ser	Asp	Phe	Leu	Met	Gln	Val	
			120					125					130			
GAT	GGA	AAA	CCC	AAA	TGT	TGC	TTC	TTT	AAA	TTT	AGC	TCT	AAA	ATA	CAA	410
Asp	Gly	Lys	Pro	Lys	Cys	Cys	Phe	Phe	Lys	Phe	Ser	Ser	Lys	Ile	Gln	
		135					140					145				
TAC	AAT	AAA	GTG	GTA	AAG	GCC	CAA	CTA	TGG	ATA	TAT	TTG	AGA	CCC	GTC	455
Tyr	Asn	Lys	Val	Val	Lys	Ala	Gln	Leu	Trp	Ile	Tyr	Leu	Arg	Pro	Val	
	150					155					160					
GAG	ACT	CCT	ACA	ACA	GTG	TTT	GTG	CAA	ATC	CTG	AGA	CTC	ATC	AAA	CCT	500
Glu	Thr	Pro	Thr	Thr	Val	Phe	Val	Gln	Ile	Leu	Arg	Leu	Ile	Lys	Pro	
	165				170					175					180	
ATG	AAA	GAC	GGT	ACA	AGG	TAT	ACT	GGA	ATC	CGA	TCT	CTG	AAA	CTT	GAC	545
Met	Lys	Asp	Gly	Thr	Arg	Tyr	Thr	Gly	Ile	Arg	Ser	Leu	Lys	Leu	Asp	
				185					190					195		
ATG	AAC	CCA	GGC	ACT	GGT	ATT	TGG	CAG	AGC	ATT	GAT	GTG	AAG	ACA	GTG	590
Met	Ser	Pro	Gly	Thr	Gly	Ile	Trp	Gln	Ser	Ile	Asp	Val	Lys	Thr	Val	
			200					205					210			

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TTG CAA AAT TGG CTC AAA CAA CCT GAA TCC AAC TTA GGC ATT GAA ATA	635
Leu Gln Asn Trp Leu Lys Gln Pro Glu Ser Asn Leu Gly Ile Glu Ile	
215 220 225	
AAA GCT TTA GAT GAG AAT GGT CAT GAT CTT GCT GTA ACC TTC CCA GGA	680
Lys Ala Leu Asp Glu Asn Gly His Asp Leu Ala Val Thr Phe Pro Gly	
230 235 240	
CCA GGA GAA GAT GGG CTG AAT CCC TTT TTA GAG GTC AAG GTA ACA GAC	725
Pro Gly Glu Asp Gly Leu Asn Pro Phe Leu Glu Val Lys Val Thr Asp	
245 250 255 260	
ACA CCC AAA AGA TCC AGA AGG GAT TTT GGT CTT GAC TGT GAT GAG CAC	770
Thr Pro Lys Arg Ser Arg Arg Asp Phe Gly Leu Asp Cys Asp Glu His	
265 270 275	
TCA ACA GAA TCG CGA TGC TGT CGT TAC CCT CTA ACT GTG GAT TTT GAA	815
Ser Thr Glu Ser Arg Cys Cys Arg Tyr Pro Leu Thr Val Asp Phe Glu	
280 285 290	
GCT CTT GGA TGG GAT TGG ATT ATC GCT CCT AAA AGA TAT AAG GCC AAT	860
Ala Phe Gly Trp Asp Trp Ile Ile Ala Pro Lys Arg Tyr Lys Ala Asn	
295 300 305	
TAC TGC TCT GGA GAG TGT GAA TTT GTA TTT TTA CAA AAA TAT CCT CAT	905
Tyr Cys Ser Gly Glu Cys Glu Phe Val Phe Leu Gln Lys Tyr Pro His	
310 315 320	
ACT CAT CTG GTA CAC CAA GCA AAC CCC AGA GGT TCA GCA GGC CCT TGC	950
Thr His Leu Val His Gln Ala Asn Pro Arg Gly Ser Ala Gly Pro Cys	
325 330 335 340	
TGT ACT CCC ACA AAG ATG TCT CCA ATT AAT ATG CTA TAT TTT AAT GGC	995
Cys Thr Pro Thr Lys Met Ser Pro Ile Asn Met Leu Tyr Phe Asn Gly	
345 350 355	
AAA GAA CAA ATA ATA TAT GGG AAA ATT CCA GCC ATG GTA GTA GAC CGC	1040
Lys Glu Gln Ile Ile Tyr Gly Lys Ile Pro Ala Met Val Val Asp Arg	
360 365 370	
TGC GGG TGC TCA TGA	1055
Cys Gly Cys Ser	
375	

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 376 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vii) IMMEDIATE SOURCE:

- (B) CLONE: Baboon GDF-8

(ix) FEATURE:

- (A) NAME/KEY: Protein
- (B) LOCATION: 1...376
- (D) OTHER:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

Met Gln Lys
1

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Leu Gln Leu Cys Val Tyr Ile Tyr Leu Phe Met Leu Ile Val Ala Gly
 5          10          15          20
Pro Val Asp Leu Asn Glu Asn Ser Glu Gln Lys Glu Asn Val Glu Lys
          25          30          35
Glu Gly Leu Cys Asn Ala Cys Thr Trp Arg Gln Asn Thr Lys Ser Ser
          40          45          50
Arg Ile Glu Ala Ile Lys Ile Gln Ile Leu Ser Lys Leu Arg Leu Glu
          55          60          65
Thr Ala Pro Asn Ile Ser Lys Asp Ala Ile Arg Gln Leu Leu Pro Lys
          70          75          80
Ala Pro Pro Leu Arg Glu Leu Ile Asp Gln Tyr Asp Val Gln Arg Asp
          85          90          95
Asp Ser Ser Asp Gly Ser Leu Glu Asp Asp Asp Tyr His Ala Thr Thr
          105          110          115
Glu Thr Ile Ile Thr Met Pro Thr Glu Ser Asp Phe Leu Met Gln Val
          120          125          130
Asp Gly Lys Pro Lys Cys Cys Phe Phe Lys Phe Ser Ser Lys Ile Gln
          135          140          145
Tyr Asn Lys Val Val Lys Ala Gln Leu Trp Ile Tyr Leu Arg Pro Val
          150          155          160
Glu Thr Pro Thr Thr Val Phe Val Gln Ile Leu Arg Leu Ile Lys Pro
          165          170          175
Met Lys Asp Gly Thr Arg Tyr Thr Gly Ile Arg Ser Leu Lys Leu Asp
          185          190          195
Met Ser Pro Gly Thr Gly Ile Trp Gln Ser Ile Asp Val Lys Thr Val
          200          205          210
Leu Gln Asn Trp Leu Lys Gln Pro Glu Ser Asn Leu Gly Ile Glu Ile
          215          220          225
Lys Ala Leu Asp Glu Asn Gly His Asp Leu Ala Val Thr Phe Pro Gly
          230          235          240
Pro Gly Glu Asp Gly Leu Asn Pro Phe Leu Glu Val Lys Val Thr Asp
          245          250          255
Thr Pro Lys Arg Ser Arg Arg Asp Phe Gly Leu Asp Cys Asp Glu His
          265          270          275
Ser Thr Glu Ser Arg Cys Cys Arg Tyr Pro Leu Thr Val Asp Phe Glu
          280          285          290
Ala Phe Gly Trp Asp Trp Ile Ile Ala Pro Lys Arg Tyr Lys Ala Asn
          295          300          305
Tyr Cys Ser Gly Glu Cys Glu Phe Val Phe Leu Gln Lys Tyr Pro His
          310          315          320
Thr His Leu Val His Gln Ala Asn Pro Arg Gly Ser Ala Gly Pro Cys
          325          330          335
Cys Thr Pro Thr Lys Met Ser Pro Ile Asn Met Leu Tyr Phe Asn Gly
          345          350          355
Lys Glu Gln Ile Ile Tyr Gly Lys Ile Pro Ala Met Val Val Asp Arg
          360          365          370
Cys Gly Cys Ser
          375

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(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1055 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: genomic DNA

(vii) IMMEDIATE SOURCE:

- (B) CLONE: Bovine GDF-8

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1...1055

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

	ATG CAA AAA	9
	Met Gln Lys	
	1	
CTG CAA ATC TCT GTT TAT ATT TAC CTA TTT ATG CTG ATT GTT GCT GGC	54	
Leu Gln Ile Ser Val Tyr Ile Tyr Leu Phe Met Leu Ile Val Ala Gly		
5 10 15 20		
CCA GTG GAT CTG AAT GAG AAC AGC GAG CAG AAG GAA AAT GTG GAA AAA	95	
Pro Val Asp Leu Asn Glu Asn Ser Glu Gln Lys Glu Asn Val Glu Lys		
25 30 35		
GAG GGG CTG TGT AAT GCA TGT TTG TGG AGG GAA AAC ACT ACA TCG TCA	140	
Glu Gly Leu Cys Asn Ala Cys Leu Trp Arg Glu Asn Thr Thr Ser Ser		
40 45 50		
AGA CTA GAA GCC ATA AAA ATC CAA ATC CTC AGT AAA CTT CGC CTG GAA	185	
Arg Leu Glu Ala Ile Lys Ile Gln Ile Leu Ser Lys Leu Arg Leu Glu		
55 60 65		
ACA GCT CCT AAC ATC AGC AAA GAT GCT ATC AGA CAA CTT TTG CCC AAG	230	
Thr Ala Pro Asn Ile Ser Lys Asp Ala Ile Arg Gln Leu Leu Pro Lys		
70 75 80		
GCT CCT CCA CTC CTG GAA CTG ATT GAT CAG TTC GAT GTC CAG AGA GAT	275	
Ala Pro Pro Leu Leu Glu Leu Ile Asp Gln Phe Asp Val Gln Arg Asp		
85 90 95 100		
GCC AGC AGT GAC GGC TCC TTG GAA GAC GAT GAC TAC CAC GCC AGG ACG	320	
Ala Ser Ser Asp Gly Ser Leu Glu Asp Asp Asp Tyr His Ala Arg Thr		
105 110 115		
GAA ACG GTC ATT ACC ATG CCC ACG GAG TCT GAT CTT CTA ACG CAA GTG	365	
Glu Thr Val Ile Thr Met Pro Thr Glu Ser Asp Leu Leu Thr Gln Val		
120 125 130		
GAA GGA AAA CCC AAA TGT TGC TTC TTT AAA TTT AGC TCT AAG ATA CAA	410	
Glu Gly Lys Pro Lys Cys Cys Phe Phe Lys Phe Ser Ser Lys Ile Gln		
135 140 145		
TAC AAT AAA CTA GTA AAG GCC CAA CTG TGG ATA TAT CTG AGG CCT GTC	455	
Tyr Asn Lys Leu Val Lys Ala Gln Leu Trp Ile Tyr Leu Arg Pro Val		
150 155 160		
AAG ACT CCT GCG ACA GTG TTT GTG CAA ATC CTG AGA CTC ATC AAA CCC	500	
Glu Thr Pro Thr Ala Val Phe Val Gln Ile Leu Arg Leu Ile Lys Pro		
165 170 175 180		
ATG AAA GAC GGT ACA AGG TAT ACT GGA ATC CGA TCT CTG AAA CTT GAC	545	
Met Lys Asp Gly Thr Arg Tyr Thr Gly Ile Arg Ser Leu Lys Leu Asp		
185 190 195		
ATG AAC CCA GGC ACT GGT ATT TGG CAG AGC ATT GAT GTG AAG ACA GTG	590	
Met Ser Pro Gly Thr Gly Ile Trp Gln Ser Ile Asp Val Lys Thr Val		
200 205 210		
TTG CAG AAC TGG CTC AAA CAA CCT GAA TCC AAC TTA GGC ATT GAA ATC	635	
Leu Gln Asn Trp Leu Lys Gln Pro Glu Ser Asn Leu Gly Ile Glu Ile		
215 220 225		

[illegible]

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 119 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(v) FRAGMENT TYPE: internal

													Met	Gln	Lys	
													1			
Leu	Gln	Ile	Ser	Val	Tyr	Ile	Tyr	Leu	Phe	Met	Leu	Ile	Val	Ala	Gly	
5					10					15					20	
Pro	Val	Asp	Leu	Asn	Glu	Asn	Ser	Glu	Gln	Lys	Glu	Asn	Val	Glu	Lys	
				25					30					35		
Glu	Gly	Leu	Cys	Asn	Ala	Cys	Leu	Trp	Arg	Glu	Asn	Thr	Thr	Ser	Ser	
			40					45					50			
Arg	Leu	Glu	Ala	Ile	Lys	Ile	Gln	Ile	Leu	Ser	Lys	Leu	Arg	Leu	Glu	
		55					60					65				
Thr	Ala	Pro	Asn	Ile	Ser	Lys	Asp	Ala	Ile	Arg	Gln	Leu	Leu	Pro	Lys	
	70					75					80					

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Ala Pro Pro Leu Leu Glu Leu Ile Asp Gln Phe Asp Val Gln Arg Asp
 85          90          95          100
Ala Ser Ser Asp Gly Ser Leu Glu Asp Asp Tyr His Ala Arg Thr
          105          110          115
Glu Thr Val Ile Thr Met Pro Thr Glu Ser Asp Leu Leu Thr Gln Val
          120          125          130
Glu Gly Lys Pro Lys Cys Cys Phe Phe Lys Phe Ser Ser Lys Ile Gln
          135          140          145
Tyr Asn Lys Leu Val Lys Ala Gln Leu Trp Ile Tyr Leu Arg Pro Val
          150          155          160
Glu Thr Pro Thr Ala Val Phe Val Gln Ile Leu Arg Leu Ile Lys Pro
165          170          175          180
Met Lys Asp Gly Thr Arg Tyr Thr Gly Ile Arg Ser Leu Lys Leu Asp
          185          190          195
Met Ser Pro Gly Thr Gly Ile Trp Gln Ser Ile Asp Val Lys Thr Val
          200          205          21
Leu Gln Asn Trp Leu Lys Gln Pro Glu Ser Asn Leu Gly Ile Glu Ile
          215          220          225
Lys Ala Leu Asp Glu Asn Gly His Asp Leu Ala Val Thr Phe Pro Glu
230          235          240
Pro Gly Glu Asp Gly Leu Thr Pro Phe Leu Glu Val Lys Val Thr Asp
245          250          255          260
Thr Pro Lys Arg Ser Arg Arg Asp Phe Gly Leu Asp Cys Asp Glu His
          265          270          275
Ser Thr Glu Ser Arg Cys Cys Arg Tyr Pro Leu Thr Val Asp Phe Glu
          280          285          290
Ala Phe Gly Trp Asp Trp Ile Ile Ala Pro Lys Arg Tyr Lys Ala Asn
295          300          305
Tyr Cys Ser Gly Glu Cys Glu Phe Val Phe Leu Gln Lys Tyr Pro His
310          315          320
Thr His Leu Val His Gln Ala Asn Pro Arg Gly Ser Ala Gly Pro Cys
325          330          335          340
Cys Thr Pro Thr Lys Met Ser Pro Ile Asn Met Leu Tyr Phe Asn Gly
          345          350          355
Lys Glu Gln Ile Ile Tyr Gly Lys Ile Pro Ala Met Val Val Asp Arg
          360          365          370
Cys Gly Cys Ser
          375

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(2) INFORMATION FOR SEQ ID NO:22:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 1055 base pairs
 (B) TYPE: nucleic acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Genomic DNA

- (vii) IMMEDIATE SOURCE:
 (B) CLONE: Chicken GDF-8

- (ix) FEATURE:
 (A) NAME/KEY: CDS
 (B) LOCATION: 1...1055
 (D) OTHER:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

ATG CAA AAG
 Met Gln Lys
 1

9

CTG GCA GTC TAT GTT TAT ATT TAC CTG TTC ATG CAG ATC GCG GTT GAT
 Leu Ala Val Tyr Val Tyr Ile Tyr Leu Phe Met Gln Ile Ala Val Asp
 5 10 15 20

54

CCG	GTG	GCT	CTG	GAT	GGC	AGT	AGT	CAG	CCC	ACA	GAG	AAC	GCT	GAA	AAA	95
Pro	Val	Ala	Leu	Asp 25	Gly	Ser	Ser	Glu	Gln 30	Lys	Glu	Asn	Val	Glu 35	Lys	
GAC	GGA	CTG	TGC	AAT	GCT	TGT	ACG	TGG	AGA	CAG	AAT	ACA	AAA	TCC	TCC	140
Glu	Gly	Leu	Cys 40	Asn	Ala	Cys	Thr	Trp 45	Arg	Gln	Asn	Thr	Lys 50	Ser	Ser	
AGA	ATA	GAA	GCC	ATA	AAA	ATT	CAA	ATC	CTC	AGC	AAA	CTG	CGC	CTG	GAA	185
Arg	Ile	Glu 55	Ala	Ile	Lys	Ile	Gln 60	Ile	Leu	Ser	Lys	Leu 65	Arg	Leu	Glu	
CAA	GCA	CCT	AAC	ATT	AGC	AGG	GAC	GTT	ATT	AAG	CAG	CTT	TTA	CCC	AAA	230
Gln	Ala 70	Pro	Asn	Ile	Ser	Arg 75	Asp	Val	Ile	Lys	Gln 80	Leu	Leu	Pro	Lys	
GCT	CCT	CCA	CTG	CAG	GAA	CTG	ATT	GAT	CAG	TAT	GAT	GTC	CAG	AGG	GAC	275
Ala 85	Pro	Pro	Leu	Gln 90	Glu	Leu	Ile	Asp	Gln 95	Tyr	Asp	Val	Gln	Arg 100	Asp	
GAC	AGT	AGC	GAT	GGC	TCT	TTG	GAA	GAC	GAT	GAC	TAT	CAT	GCC	ACA	ACC	320
Asp	Ser	Ser	Asp	Gly 105	Ser	Leu	Glu	Asp	Asp 110	Asp	Tyr	His	Ala	Thr 115	Thr	
GAG	ACG	ATT	ATC	ACA	ATG	CCT	ACG	GAG	TCT	GAT	TTT	CTT	GTA	CAA	ATG	365
Glu	Thr	Ile	Ile 120	Thr	Met	Pro	Thr	Glu 125	Ser	Asp	Phe	Leu	Val 130	Gln	Met	
GAG	GGA	AAA	CCA	AAA	TGT	TGC	TTC	TTT	AAG	TTT	AGC	TCT	AAA	ATA	CAA	410
Glu	Gly	Lys 135	Pro	Lys	Cys	Cys	Phe 140	Phe	Lys	Phe	Ser	Ser 145	Lys	Ile	Gln	
TAT	AAC	AAA	GTA	GTA	AAG	GCA	CAA	TTA	TGG	ATA	TAC	TTG	AGG	CAA	GTC	455
Tyr	Asn 150	Lys	Val	Val	Lys	Ala 155	Gln	Leu	Trp	Ile	Tyr 160	Leu	Arg	Gln	Val	
CAA	AAA	CCT	ACA	ACG	GTG	TTT	GTG	CAG	ATC	CTG	AGA	CTC	ATT	AAG	CCC	500
Gln 165	Lys	Pro	Thr	Thr 170	Val	Phe	Val	Gln	Ile 175	Leu	Arg	Leu	Ile	Lys 180	Pro	
ATG	AAA	GAC	GGT	ACA	AGA	TAT	ACT	GGA	ATT	CGA	TCT	TTG	AAA	CTT	GAC	545
Met	Lys	Asp	Gly 185	Thr	Arg	Tyr	Thr	Gly 190	Ile	Arg	Ser	Leu	Lys 195	Leu	Asp	
ATG	AAC	CCA	GGC	ACT	GGT	ATC	TGG	CAG	AGT	ATT	GAT	GTG	AAG	ACA	GTG	590
Met	Ser	Pro	Gly 200	Thr	Gly	Ile	Trp	Gln 205	Ser	Ile	Asp	Val 210	Lys	Thr	Val	
CTG	CAA	AAT	TGG	CTC	AAA	CAG	CCT	GAA	TCC	AAT	TTA	GGC	ATC	GAA	ATA	635
Leu	Gln 215	Asn	Trp	Leu	Lys	Gln	Pro 220	Glu	Ser	Asn	Leu	Gly 225	Ile	Glu	Ile	
AAA	GCT	TTT	GAT	GAG	ACT	GGA	CGA	GAT	CTT	GCT	GTC	ACA	TTC	CCA	GGA	680
Lys	Ala 230	Phe	Asp	Glu	Thr	Gly 235	Arg	Asp	Leu	Ala	Val 240	Thr	Phe	Pro	Gly	
CCA	GGA	GAA	GAT	GGA	TTG	AAC	CCA	TTT	TTA	GAG	GTC	AGA	GTT	ACA	GAC	725
Pro 245	Gly	Glu	Asp	Gly 250	Leu	Asn	Pro	Phe	Leu	Glu 255	Val	Arg	Val	Thr	Asp 260	
ACA	CCG	AAA	CGG	TCC	CGC	AGA	GAT	TTT	GGC	CTT	GAC	TGT	GAT	GAG	CAC	770
Thr	Pro	Lys 265	Arg	Ser 265	Arg	Arg	Asp	Phe 270	Gly 270	Leu	Asp	Cys	Asp 275	Glu 275	His	

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TCA ACG GAA TCC CGA TGT TGT CGC TAC CCG CTG ACA GTG GAT TTC GAA	815
Ser Thr Glu Ser Arg Cys Cys Arg Tyr Pro Leu Thr Val Asp Phe Glu	
280 285 290	
GCT TTT GGA TGG GAC TGG ATT ATA GCA CCT AAA AGA TAC AAA GCC AAT	860
Ala Phe Gly Trp Asp Trp Ile Ile Ala Pro Lys Arg Tyr Lys Ala Asn	
295 300 305	
TAC TGC TCC GGA GAA TGC GAA TTT GTG TTT CTA CAG AAA TAC CCG CAC	905
Tyr Cys Ser Gly Glu Cys Glu Phe Val Phe Leu Gln Lys Tyr Pro His	
310 315 320	
ACT CAC CTG GTA CAC CAA GCA AAT CCC AGA GGC TCA GCA GGC CCT TGC	950
Thr His Leu Val His Gln Ala Asn Pro Arg Gly Ser Ala Gly Pro Cys	
325 330 335 340	
TGC ACA CCC ACC AAG ATG TCC CCT ATA AAC ATG CTG TAT TTC AAT GGA	995
Cys Thr Pro Thr Lys Met Ser Pro Ile Asn Met Leu Tyr Phe Asn Gly	
345 350 355	
AAA GAA CAA ATA TAT GGA AAG ATA CCA GCC ATG GTT GTA GAT CGT	1040
Lys Glu Gln Ile Ile Tyr Gly Lys Ile Pro Ala Met Val Val Asp Arg	
360 365 370	
TGC GGG TGC TCA TGA	1055
Cys Gly Cys Ser	
375	

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 376 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(v) FRAGMENT TYPE: internal

(vii) IMMEDIATE SOURCE:

- (B) CLONE: Chicken GDF-8

(ix) FEATURE:

- (A) NAME/KEY: Protein
- (B) LOCATION: 1..376
- (D) OTHER:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

												Met	Gln	Lys	
												1			
Leu	Ala	Val	Tyr	Val	Tyr	Ile	Tyr	Leu	Phe	Met	Gln	Ile	Ala	Val	Asp
5					10				15					20	
Pro	Val	Ala	Leu	Asp	Gly	Ser	Ser	Glu	Gln	Lys	Glu	Asn	Val	Glu	Lys
				25				30						35	
Glu	Gly	Leu	Cys	Asn	Ala	Cys	Thr	Trp	Arg	Gln	Asn	Thr	Lys	Ser	Ser
			40				45					50			
Arg	Ile	Glu	Ala	Ile	Lys	Ile	Gln	Ile	Leu	Ser	Lys	Leu	Arg	Leu	Glu
	55					60					65				
Gln	Ala	Pro	Asn	Ile	Ser	Arg	Asp	Val	Ile	Lys	Gln	Leu	Leu	Pro	Lys
	70				75					80					
Ala	Pro	Pro	Leu	Gln	Glu	Leu	Ile	Asp	Gln	Tyr	Asp	Val	Gln	Arg	Asp
85				90				95						100	
Asp	Ser	Ser	Asp	Gly	Ser	Leu	Glu	Asp	Asp	Asp	Tyr	His	Ala	Thr	Thr
			105					110						11	
Glu	Thr	Ile	Ile	Thr	Met	Pro	Thr	Glu	Ser	Asp	Phe	Leu	Val	Gln	Met
			120				125						130		

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[illegible]

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1276 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Genomic DNA

(vii) IMMEDIATE SOURCE:

- (B) CLONE: Rat GDF-8

(ix) FEATURE:

- (A) NAME/KEY: CDS
(B) LOCATION: 1...1276
(D) OTHER:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

ATG ATT CAA AAA 115
Met Ile Gln Lys
1

CCG CAA ATG TAT GTT TAT ATT TAC CTG TTT GTG CTG ATT GCT GCT GGC 163
Pro Gln Met Tyr Val Tyr Ile Tyr Leu Phe Val Leu Ile Ala Ala Gly
5 10 15 20

CCA GTG GAT CTA AAT GAG GAC AGT GAG AGA GAG GCG AAT GTG GAA AAA 211
Pro Val Asp Leu Asn Glu Asp Ser Glu Arg Glu Ala Asn Val Glu Lys
25 30 35

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GAG	GGG	CTG	TGT	AAT	GCG	TGT	GCG	TGG	AGA	CAA	AAC	ACA	AGG	TAC	TCC	259
Glu	Gly	Leu	Cys	Asn	Ala	Cys	Ala	Trp	Arg	Gln	Asn	Thr	Arg	Tyr	Ser	
		40						45					50			
AGA	ATA	GAA	GCC	ATA	AAA	ATT	CAA	ATC	CTC	AGT	AAA	CTC	CGC	CTG	GAA	307
Arg	Ile	Glu	Ala	Ile	Lys	Ile	Gln	Ile	Leu	Ser	Lys	Leu	Arg	Leu	Glu	
		55					60					65				
ACA	GCG	CCT	AAC	ATC	AGC	AAA	GAT	GCT	ATA	AGA	CAA	CTT	CTG	CCC	AGA	355
Thr	Ala	Pro	Asn	Ile	Ser	Lys	Asp	Ala	Ile	Arg	Gln	Leu	Leu	Pro	Arg	
	70					75					80					
GCG	CCT	CCA	CTC	CGG	GAA	CTG	ATC	GAT	CAG	TAC	GAC	GTC	CAG	AGG	GAT	403
Ala	Pro	Pro	Leu	Arg	Glu	Leu	Ile	Asp	Gln	Tyr	Asp	Val	Gln	Arg	Asp	
	85				90					95					100	
GAC	AGC	AGT	GAC	GGC	TCT	TTG	GAA	GAT	GAC	GAT	TAT	CAC	GCT	ACC	ACG	451
Asp	Ser	Ser	Asp	Gly	Ser	Leu	Glu	Asp	Asp	Asp	Tyr	His	Ala	Thr	Thr	
				105					110					115		
GAA	ACA	ATC	ATT	ACC	ATG	CCT	ACC	GAG	TCT	GAC	TTT	CTA	ATG	CAA	GCG	499
Glu	Thr	Ile	Ile	Thr	Met	Pro	Thr	Glu	Ser	Asp	Phe	Leu	Met	Gln	Ala	
			120					125					130			
GAT	GGA	AAG	CCC	AAA	TGT	TGC	TTT	TTT	AAA	TTT	AGC	TCT	AAA	ATA	CAG	547
Asp	Gly	Lys	Pro	Lys	Cys	Cys	Phe	Phe	Lys	Phe	Ser	Ser	Lys	Ile	Gln	
		135					140					145				
TAC	AAC	AAA	GTG	GTA	AAG	GCC	CAG	CTG	TGG	ATA	TAT	CTG	AGA	GCC	GTC	595
Tyr	Asn	Lys	Val	Val	Lys	Ala	Gln	Leu	Trp	Ile	Tyr	Leu	Arg	Ala	Val	
	150					155					160					
AAG	ACT	CCT	ACA	ACA	GTG	TTT	GTG	CAA	ATC	CTG	AGA	CTC	ATC	AAA	CCC	643
Lys	Thr	Pro	Thr	Thr	Val	Phe	Val	Gln	Ile	Leu	Arg	Leu	Ile	Lys	Pro	
	165				170					175					180	
ATG	AAA	GAC	GGT	ACA	AGG	TAT	ACC	GGA	ATC	CGA	TCT	CTG	AAA	CTT	GAC	691
Met	Lys	Asp	Gly	Thr	Arg	Tyr	Thr	Gly	Ile	Arg	Ser	Leu	Lys	Leu	Asp	
				185				190						195		
ATG	AGC	CCA	GGC	ACT	GGT	ATT	TGG	CAG	AGT	ATT	GAT	GTG	AAG	ACA	GTG	739
Met	Ser	Pro	Gly	Thr	Gly	Ile	Trp	Gln	Ser	Ile	Asp	Val	Lys	Thr	Val	
		200						205					210			
TTG	CAA	AAT	TGG	CTC	AAA	CAG	CCT	GAA	TCC	AAC	TTA	GGC	ATT	GAA	ATC	787
Leu	Gln	Asn	Trp	Leu	Lys	Gln	Pro	Glu	Ser	Asn	Leu	Gly	Ile	Glu	Ile	
		215					220					225				
AAA	GCT	TTG	GAT	GAG	AAT	GGG	CAT	GAT	CTT	GCT	GTA	ACC	TTC	CCA	GGA	835
Lys	Ala	Leu	Asp	Glu	Asn	Gly	His	Asp	Leu	Ala	Val	Thr	Phe	Pro	Gly	
	230					235					240					
CCA	GGA	GAA	GAT	GGG	CTG	AAT	CCC	TTT	TTA	GAA	GTC	AAA	GTA	ACA	GAC	883
Pro	Gly	Glu	Asp	Gly	Leu	Asn	Pro	Phe	Leu	Glu	Val	Lys	Val	Thr	Asp	
	245				250					255					260	
ACA	CCC	AAG	AGG	TCC	CGG	AGA	GAC	TTT	GGG	CTT	GAC	TGC	GAT	GAA	CAC	931
Thr	Pro	Lys	Arg	Ser	Arg	Arg	Asp	Phe	Gly	Leu	Asp	Cys	Asp	Glu	His	
				265					270					275		
TCC	ACG	GAA	TCG	CGG	TGC	TGT	CGC	TAC	CCC	CTC	ACG	GTC	GAT	TTC	GAA	979
Ser	Thr	Glu	Ser	Arg	Cys	Cys	Arg	Tyr	Pro	Leu	Thr	Val	Asp	Phe	Glu	
			280					285					290			

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GCC TTT GGA TGG GAC TGG ATT ATT GCA CCC AAA AGA TAT AAG GCT AAT	1027
Ala Phe Gly Trp Asp Trp Ile Ile Ala Pro Lys Arg Tyr Lys Ala Asn	
295 300 305	
TAC TGC TCT GGA GAG TGT GAA TTT GTG TTC TTA CAA AAA TAT CCG CAT	1075
Tyr Cys Ser Gly Glu Cys Glu Phe Val Phe Leu Gln Lys Tyr Pro His	
310 315 320	
ACT CAT CTT GTG CAC CAA GCA AAC CCC AGA GGC TCG GCA GGC CCT TGC	1123
Thr His Leu Val His Gln Ala Asn Pro Arg Gly Ser Ala Gly Pro Cys	
325 330 335 340	
TGC ACG CCA ACA AAA ATG TCT CCC ATT AAT ATG CTA TAT TTT AAT GGC	1171
Cys Thr Pro Thr Lys Met Ser Pro Ile Asn Met Leu Tyr Phe Asn Gly	
345 350 355	
AAA GAA CAA ATA ATA TAT GGG AAA ATT CCA GCC ATG GTA GTA GAC CGG	1219
Lys Glu Gln Ile Ile Tyr Gly Lys Ile Pro Ala Met Val Val Asp Arg	
360 365 370	
TGT GGG TGC TCG TGA	1276
Cys Gly Cys Ser	
375	

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 376 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vii) IMMEDIATE SOURCE:

- (B) CLONE: Rat GDF-8

(ix) FEATURE:

- (A) NAME/KEY: Protein
 (B) LOCATION: 1..376
 (D) OTHER:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

															Met	Ile	Gln	Lys	
															1				
Pro	Gln	Met	Tyr	Val	Tyr	Ile	Tyr	Leu	Phe	Val	Leu	Ile	Ala	Ala	Gly				
5					10					15					20				
Pro	Val	Asp	Leu	Asn	Glu	Asp	Ser	Glu	Arg	Glu	Ala	Asn	Val	Glu	Lys				
				25					30						35				
Glu	Gly	Leu	Cys	Asn	Ala	Cys	Ala	Trp	Arg	Gln	Asn	Thr	Arg	Tyr	Ser				
			40					45					50						
Arg	Ile	Glu	Ala	Ile	Lys	Ile	Gln	Ile	Leu	Ser	Lys	Leu	Arg	Leu	Glu				
		55					60					65							
Thr	Ala	Pro	Asn	Ile	Ser	Lys	Asp	Ala	Ile	Arg	Gln	Leu	Leu	Pro	Arg				
	70					75					80								
Ala	Pro	Pro	Leu	Arg	Glu	Leu	Ile	Asp	Gln	Tyr	Asp	Val	Gln	Arg	Asp				
				90					95						100				
Asp	Ser	Ser	Asp	Gly	Ser	Leu	Glu	Asp	Asp	Asp	Tyr	His	Ala	Thr	Thr				
				105					110						115				
Glu	Thr	Ile	Ile	Thr	Met	Pro	Thr	Glu	Ser	Asp	Phe	Leu	Met	Gln	Ala				
				120				125					130						
Asp	Gly	Lys	Pro	Lys	Cys	Cys	Phe	Phe	Lys	Phe	Ser	Ser	Lys	Ile	Gln				
		135					140					145							
Tyr	Asn	Lys	Val	Val	Lys	Ala	Gln	Leu	Trp	Ile	Tyr	Leu	Arg	Ala	Val				
		150				155						160							

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Lys Thr Pro Thr Thr Val Phe Val Gln Ile Leu Arg Leu Ile Lys Pro
 165 170 175 180
 Met Lys Asp Gly Thr Arg Tyr Thr Gly Ile Arg Ser Leu Lys Leu Asp
 185 190 195
 Met Ser Pro Gly Thr Gly Ile Trp Gln Ser Ile Asp Val Lys Thr Val
 200 205 210
 Leu Gln Asn Trp Leu Lys Gln Pro Glu Ser Asn Leu Gly Ile Glu Ile
 215 220 225
 Lys Ala Leu Asp Glu Asn Gly His Asp Leu Ala Val Thr Phe Pro Gly
 230 235 240
 Pro Gly Glu Asp Gly Leu Asn Pro Phe Leu Glu Val Lys Val Thr Asp
 245 250 255 260
 Thr Pro Lys Arg Ser Arg Arg Asp Phe Gly Leu Asp Cys Asp Glu His
 265 270 275
 Ser Thr Glu Ser Arg Cys Cys Arg Tyr Pro Leu Thr Val Asp Phe Glu
 280 285 290
 Ala Phe Gly Trp Asp Trp Ile Ile Ala Pro Lys Arg Tyr Lys Ala Asn
 295 300 305
 Tyr Cys Ser Gly Glu Cys Glu Phe Val Phe Leu Gln Lys Tyr Pro His
 310 315 320
 Thr His Leu Val His Gln Ala Asn Pro Arg Gly Ser Ala Gly Pro Cys
 325 330 335 340
 Cys Thr Pro Thr Lys Met Ser Pro Ile Asn Met Leu Tyr Phe Asn Gly
 345 350 355
 Lys Glu Gln Ile Ile Tyr Gly Lys Ile Pro Ala Met Val Val Asp Arg
 360 365 370
 Cys Gly Cys Ser
 375

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1055 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Genomic DNA

(vii) IMMEDIATE SOURCE:

- (B) CLONE: Turkey GDF-8

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1...1055
- (D) OTHER:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

	ATG CAA AAG	9
	Met Gln Lys	
	1	
CTA GCA GTC TAT GTT TAT ATT TAC CTG TTC ATG CAG ATT TTA GTT CAT	54	
Leu Ala Val Tyr Val Tyr Ile Tyr Leu Phe Met Gln Ile Leu Val His		
5 10 15 20		
CCG GTG GCT CTT GAT GGC AGT AGT CAG CCC ACA GAG AAC GCT GAA AAA	95	
Pro Val Ala Leu Asp Gly Ser Ser Glu Gln Lys Glu Asn Val Glu Lys		
25 30 35		
GAC GGA CTG TGC AAT GCT TGC ACG TGG AGA CAG AAT ACT AAA TCC TCC	140	
Glu Gly Leu Cys Asn Ala Cys Thr Trp Arg Gln Asn Thr Lys Ser Ser		
40 45 50		

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AGA Arg	ATA Ile	GAA Glu	GCC Ala	ATA Ile	AAA Lys	ATT Ile	CAA Gln	ATC Ile	CTC Leu	AGC Ser	AAA Lys	CTG Leu	CGC Arg	CTG Leu	GAA Glu	185
		55					60					65				
CAA Gln	GCA Ala	CCT Pro	AAC Asn	ATT Ile	AGC Ser	AGG Arg	GAC Asp	GTT Val	ATT Ile	AAA Lys	CAA Gln	CTT Leu	TTA Leu	CCC Pro	AAA Lys	230
	70					75					80					
GCT Ala	CCT Pro	CCG Pro	CTG Leu	CAG Gln	GAA Glu	CTG Leu	ATT Ile	GAT Asp	CAG Gln	TAT Tyr	GAC Asp	GTC Val	CAG Gln	AGA Arg	GAC Asp	275
85					90					95					100	
GAC Asp	AGT Ser	AGC Ser	GAT Asp	GGC Gly	TCT Ser	TTG Leu	GAA Glu	GAC Asp	GAT Asp	GAC Asp	TAT Tyr	CAT His	GCC Ala	ACA Thr	ACC Thr	320
				105					110					115		
GAA Glu	ACG Thr	ATT Ile	ATC Ile	ACA Thr	ATG Met	CCT Pro	ACG Thr	GAG Glu	TCT Ser	GAT Asp	TTT Phe	CTT Leu	GTA Val	CAA Gln	ATG Met	365
				120				125					130			
GAG Glu	GGA Gly	AAA Lys	CCA Pro	AAA Lys	TGT Cys	TGC Cys	TTC Phe	TTT Phe	AAG Lys	TTT Phe	AGC Ser	TCT Ser	AAA Lys	ATA Ile	CAA Gln	410
		135					140					145				
TAT Tyr	AAC Asn	AAA Lys	GTA Val	GTA Val	AAG Lys	GCA Ala	CAA Gln	TTA Leu	TGG Trp	ATA Ile	TAC Tyr	TTG Leu	AGG Arg	CAA Gln	GTC Val	455
	150					155					160					
CAA Gln	AAA Lys	CCT Pro	ACA Thr	ACG Thr	GTG Val	TTT Phe	GTG Val	CAG Gln	ATC Ile	CTG Leu	AGA Arg	CTC Leu	ATT Ile	AAA Lys	CCC Pro	500
165					170					175					180	
ATG Met	AAA Lys	GAC Asp	GGT Gly	ACA Thr	AGA Arg	TAT Tyr	ACT Thr	GGA Gly	ATT Ile	CGA Arg	TCT Ser	TTG Leu	AAA Lys	CTT Leu	GAC Asp	545
				185					190					195		
ATG Met	AAC Ser	CCA Pro	GGC Gly	ACT Thr	GGT Gly	ATC Ile	TGG Trp	CAG Gln	AGT Ser	ATT Ile	GAT Asp	GTG Val	AAG Lys	ACA Thr	GTG Val	590
			200					205				210				
TTG Leu	CAA Gln	AAT Asn	TGG Trp	CTC Leu	AAA Lys	CAG Gln	CCT Pro	GAA Glu	TCC Ser	AAT Asn	TTA Leu	GGC Gly	ATC Ile	GAA Glu	ATA Ile	635
		215					220					225				
AAA Lys	GCT Ala	TTT Phe	GAT Asp	GAG Glu	AAT Asn	GGA Gly	CGA Arg	GAT Asp	CTT Leu	GCT Ala	GTA Val	ACA Thr	TTC Phe	CCA Pro	GGA Gly	680
	230					235				240						
CCA Pro	GGT Gly	GAA Glu	GAT Asp	GGA Gly	CTG Leu	AAC Asn	CCA Pro	TTT Phe	TTA Leu	GAG Glu	GTC Val	AGA Arg	GTT Val	ACA Thr	GAC Asp	725
245					250					255					260	
ACA Thr	CCA Pro	AAA Lys	CGG Arg	TCC Ser	CGC Arg	AGA Arg	GAT Asp	TTT Phe	GGC Gly	CTT Leu	GAC Asp	TGC Cys	GAC Asp	GAG Glu	CAC His	770
				265					270					275		
TCA Ser	ACG Thr	GAA Glu	TCT Ser	CGA Arg	TGT Cys	TGT Cys	CGC Arg	TAC Tyr	CCG Pro	CTG Leu	ACA Thr	GTG Val	GAT Asp	TTT Phe	GAA Glu	815
			280					285					290			
GCT Ala	TTT Phe	GGA Gly	TGG Trp	GAC Asp	TGG Trp	ATT Ile	ATA Ile	GCA Ala	CCT Pro	AAA Lys	AGA Arg	TAC Tyr	AAA Lys	GCC Ala	AAT Asn	860
		295					300					305				

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 376 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(vii) IMMEDIATE SOURCE:
(B) CLONE: Turkey GDF-8

(A) NAME/KEY: Protein
(B) LOCATION: 1..376
(D) OTHER:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:											Met Gln Lys				
											1				
Leu	Ala	Val	Tyr	Val	Tyr	Ile	Tyr	Leu	Phe	Met	Gln	Ile	Leu	Val	His
5					10					15					20
Pro	Val	Ala	Leu	Asp	Gly	Ser	Ser	Glu	Gln	Lys	Glu	Asn	Val	Glu	Lys
				25					30					35	
Glu	Gly	Leu	Cys	Asn	Ala	Cys	Thr	Trp	Arg	Gln	Asn	Thr	Lys	Ser	Ser
			40					45					50		
Arg	Ile	Glu	Ala	Ile	Lys	Ile	Gln	Ile	Leu	Ser	Lys	Leu	Arg	Leu	Glu
		55					60					65			
Gln	Ala	Pro	Asn	Ile	Ser	Arg	Asp	Val	Ile	Lys	Gln	Leu	Leu	Pro	Lys
	70					75						80			
Ala	Pro	Pro	Leu	Gln	Glu	Leu	Ile	Asp	Gln	Tyr	Asp	Val	Gln	Arg	Asp
85					90					95					100
Asp	Ser	Ser	Asp	Gly	Ser	Leu	Glu	Asp	Asp	Asp	Tyr	His	Ala	Thr	Thr
				105					110					115	
Glu	Thr	Ile	Ile	Thr	Met	Pro	Thr	Glu	Ser	Asp	Phe	Leu	Val	Gln	Met
			120					125					130		
Glu	Gly	Lys	Pro	Lys	Cys	Cys	Phe	Phe	Lys	Phe	Ser	Ser	Lys	Ile	Gln
		135					140					145			
Tyr	Asn	Lys	Val	Val	Lys	Ala	Gln	Leu	Trp	Ile	Tyr	Leu	Arg	Gln	Val
	150					155						160			
Gln	Lys	Pro	Thr	Thr	Val	Phe	Val	Gln	Ile	Leu	Arg	Leu	Ile	Lys	Pro
165					170					175					180
Met	Lys	Asp	Gly	Thr	Arg	Tyr	Thr	Gly	Ile	Arg	Ser	Leu	Lys	Leu	Asp
			185						190					195	
Met	Ser	Pro	Gly	Thr	Gly	Ile	Trp	Gln	Ser	Ile	Asp	Val	Lys	Thr	Val
			200					205					210		

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/02511

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : C12N 5/00, 15/00, 15/09, 15/63; G01N 33/00; A61K 39/395, 48/00
US CL : 800/3, 8; 514/44; 424/130.1

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 800/3, 8; 514/44; 424/130.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 94/21681 A1 (THE JOHNS HOPKINS UNIVERSITY SCHOOL OF MEDICINE) 29 September 1994, see entire document.	1-12
A	WO 96/01845 A1 (THE JOHNS HOPKINS UNIVERSITY SCHOOL OF MEDICINE) 25 January 1996, see entire document.	1-12
A,P	WO 98/33887 A1 (THE JOHNS HOPKINS UNIVERSITY SCHOOL OF MEDICINE) 06 August 1998, see entire document	1-12
X,P	US 5,827,733 A (LEE et al) 27 October 1998, see entire document.	1-12
X	SLACK, J.M.W. Growth Control: action mouse. Current Biology. 01 August 1997, Vol. 7, No. 8, pages R467-R469, see entire document.	1-12

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
A document defining the general state of the art which is not considered to be of particular relevance	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
B earlier document published on or after the international filing date	*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
L document which may throw doubt on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*Z* document member of the same patent family
O document referring to an oral disclosure, use, exhibition or other means	
P document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search
23 APRIL 1999

Date of mailing of the international search report
13 MAY 1999

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Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/02511

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	LOVE et al. Transgenic birds by DNA microinjection. Bio/Technology. January 1994, Vol. 12, No. 1, pages 60-63. See entire document.	4-7

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/02511

B. FIELDS SEARCHED

Electronic data bases consulted (Name of data base and where practicable terms used):

APS, caplus wpids, biosis, medline.

search terms gdf-8 or growth differentiation factor 8 or myostatin, transgenic, antisense, antibodies, treat or therap, renal or kidney